SHOCK AND CIRCULATORY HOMEOSTASIS

Transactions of the Fifth Conference
November 30, December 1, and 2, 1955, Princeton, N J

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Sponsored by
JOSIAH MACY JR. FOUNDATION
New York, N Y

Library of Congress Catalog Card Number: 52 136 7
Price $4 75

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Printed in the United States of America
by Madison Printing Company Inc., Madison New Jersey

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FRANK FREMONT-SMITH, *Medical Director*
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TABLE OF CONTENTS

Fifth Conference on Shock and Circulatory Homeostasis

THE JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

DURING THE PAST FIFTEEN years the Josiah Macy Jr Foundation has organized more than twenty conference groups, each group meeting for at least two days annually over a period of five or more years Each meeting is limited to twenty five participants (members and guests) selected to represent a multidiscipline approach to some urgent problem in the field of medicine and health. The goal of this conference program is the promotion of communication, the exchange of ideas, and the stimulation of creativity among the participants The purpose of the publication of the transactions of the meetings is to share, as far as possible, the conference process with a larger audience than could participate personally in the discussions

These conferences provide an opportunity for informal give and take among the participants To further this purpose the number of presentations planned for each day is generally restricted to one or two The member or guest, selected to give such a presentation is requested not to read a paper but rather to highlight, in an informal manner some of the more interesting aspects of his or her research, with the expectation that there will be frequent interruptions by participants in the form of questions criticism or comment Such interruptions during the course of a presentation are encouraged and form an essential part of the group interchange.

The conference program has always been viewed by the Foundation as an experiment in communication in which there is room for improvement and need for frequent reappraisal Sufficient experience has already been gained to justify the conclusion that this type of conference is an effective way of improving understanding among scientists in medicine and allied disciplines of broadening perspectives of changing attitudes and of overcoming prejudices The further conclusion has been reached, as the result of this experiment, that the major obstructions to understanding among scientists lie in the resistance of human attitudes to change rather than in difficulties of technical comprehension Less extensive experience with non-scientists has indicated that the effectiveness of this type of conference is not limited to groups of scientists but will function in any group meeting where more effective

communication is the primary goal. It is also clear that the same conference technique, with minor changes, is readily adapted to small international conferences.

The style of publication of the Transactions has aroused considerable interest and some criticism. The criticism has been directed primarily to editorial permissiveness which has allowed in the final text, in some instances, too many questions, remarks or comments which, although perhaps useful during a heated discussion, seem out of context and interrupt the sequence of thought in the printed volume. A few have objected to the principle of publishing in this style and would prefer a depersonalized summary without interruptions.

The Foundation Staff and the Scientific Editors of these volumes welcome criticism and hope to profit thereby in increasing the usefulness of the Transactions to scientists and students of science in this country and abroad.

FRANK FREMONT-SMITH, M.D.
Medical Director

EPHRAIM SHORR

June 1, 1897 – January 6, 1956

Ephraim Shorr brought to the Chairmanship of the five Conferences on Shock and Circulatory Homeostasis a unique cluster of talents. His rich capacity for original and creative ideas was well disciplined by long experience with the experimental method. The range of his competence in scientific research extended from the intricate biochemical processes within living cells to the integrative functions of organs and systems which maintain the intactness of the organism as a whole.

Above all Ephraim Shorr was a great physician. He was unsparing of himself in responding to the needs of his patients and their families. His diagnostic skill and clinical judgment were outstanding. As a teacher and friend he was receptive to new ideas, kindly in criticism, and generous with encouragement. He was unswerving in his devotion to scientific and human ideals.

We who have shared the privilege of close association with Ephraim Shorr mourn our irreparable loss in his untimely death. We will always cherish the gift of his friendship and rejoice that he has lived.

FRANK FREMONT-SMITH

HEPATIC BLOOD FLOW IN EXPERIMENTAL SHOCK

STANLEY E BRADLEY
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THE HEPATIC CIRCULATION undoubtedly plays an important role in the circulatory adjustments that develop in the body following hemorrhage or trauma There is too little direct or reliable evidence available to permit more than a tentative hypothesis regarding the character and mechanisms of the changes that occur in the splanchnic vascular bed. The information yielded by morphologic and physiologic study often appears to be in conflict and successive or continuing shifts in the pattern of response complicate the picture In consequence, interpretation must be guarded and generalizations accepted with caution. At this conference my task, as I understand it is simply to present the problem, to note some of its complex features and to raise questions that seem important for further investigation. In initiating the discussion, I should like to avoid the term shock and all of the confusion that term involves but I know quite well that it is not altogether possible. I shall deal with the problem of adjustments in the splanchnic bed which are precipitated by blood loss and a fall in the total blood volume Whether these considerations are also germane to the shock that follows trauma, burns fever and so forth, is uncertain, but it seems possible that to some extent they may apply Here at once we enter an area regarding which there is little or no evidence more is needed, and a trustworthy survey must wait upon further investigation

The size of the splanchnic bed and the volume of blood lying within it and flowing through it each minute give a seemingly sound *a priori* basis for the belief that it is important in systemic hemodynamic adjustments Measurements of splanchnic blood flow following hemorrhage in dogs indicate that the blood flow diminishes but no more than would be expected on the basis of the associated fall in arterial pressure alone (1 2,3) Does this mean that vasoconstriction does not develop at all or that the various components of this complex bed are affected

differently with no net change in calculated resistance? Do hepatic arterial inflow mesenteric arterial inflow and splenic inflow behave similarly? I have no answers to these questions I hope that someone among you will

Further observations of the behavior of the splanchnic circulation suggest that vasodilation develops spontaneously within an hour or more after a single hemorrhage and that it occurs in the absence of a notable restoration or arterial pressure or cardiac output This spontaneous recovery if recovery it is must be associated with compensatory adjustments elsewhere in the body so that a further fall in blood pressure is prevented Where do these adjustments take place? Does this response imply that protracted reduction in hepatic blood supply gives rise to local alterations that induce vasodilation? Within what parts of the splanchnic vasculature is this response most marked or is it a generalized phenomenon? What is its mechanism, neural or humoral?

When hemorrhage is repeated at frequent intervals so as to prevent any effective restoration of the blood pressure level the splanchnic blood flow continues to be depressed at least to the same extent as the perfusing pressure In what manner then is the spontaneous recovery just alluded to that follows a single bleeding interfered with? Is there a shift in the splanchnic hemodynamic adjustment in more protracted hypotensive states? Do all parts of the splanchnic vascular bed behave similarly in this response? Is there, indeed vasoconstriction in some sections and vasodilation in others?

Measurements of splanchnic blood volume following hemorrhage indicate that the amount of circulating blood held within the splanchnic blood vessels decreases In this manner a considerable volume of blood is transferred from the splanchnic reservoir to the systemic circuit as a kind of autotransfusion. This phenomenon is observed in both intact and splenectomized animals and it seems probable that movement of blood from the spleen may also contribute to the shift How is this transfer brought about? Does vasoconstriction result in an active reduction in splanchnic vascular capacity or is a fall in distending pressure which results from the decrement in perfusing pressure and perhaps also from arteriolar vasoconstriction sufficient to account for the change? If this effect depends upon vasomotor adjustments how is it mediated?

Much has been said about the possibility of "pooling in the splanchnic bed as a factor conducive to the development of irreversible shock but clear-cut evidence that it actually occurs as a primary event seems so far to have eluded us The splanchnic blood volume seems to

be greatly reduced after hours of sustained hypotension. It may be argued that a greater proportion of the total blood volume is confined to the splanchnic bed but accurate data on the distribution of blood in different parts of the body at different stages in the progression to irreversibility are not yet, to my knowledge, available.

In this brief introduction I have been content to point to a number of unanswered questions regarding the role of the splanchnic circulation in the circulatory adjustments to hemorrhage or other causes of arterial hypotension. I do hope that answers to many of them will emerge from the discussion to follow

Zweifach Are you considering the visceral mass as one organ system?

Bradley I think of the splanchnic bed as that system of vessels which lies between the aorta and the hepatic vein, and this includes the vasculature of the spleen the gastrointestinal tract, the pancreas, the portal venous system, and the hepatic artery and its drainage system.

Zweifach Do you believe that all of these visceral structures necessarily undergo changes in the same direction or to the same degree?

Bradley No there is no *a priori* reason to suppose that all the components of this system should behave similarly Indeed, there is much evidence that they do not but it seems to be fragmentary in character and not as yet satisfactorily integrated

Zweifach I believe that our direct microcirculatory studies have provided pertinent information in this regard In the omentum, the circulation becomes highly ischemic following simple hemorrhage sufficient to lower the blood pressure of the anesthetized dog to from 40 to 50 mm. Hg Observations on the mesentery of the small intestine permit us to record the flow in the large veins and arteries which feed the bowel proper Both the arteries (from 150 to 250 μ) and the veins (from 200 to 400 μ) undergo extreme constriction to less than one third of the original caliber The return of blood from the bowel through the large veins is slowed to such an extent that the blood is moving only sluggishly and intermittently One of our criteria of shock has been the degree of hypotension which was necessary to interfere with the venous return of blood from the bowel by way of these large veins Irreversible hemorrhagic shock, a state which progressively becomes refractory to blood replacement does not develop unless the blood pressure is lowered sufficiently to interfere with the venous return of blood flow from the bowel

Observations on the surface of the small intestine (especially in small animals such as the rat) indicate a sluggish ischemic circulation during the initial phase of the hypotensive episode and venous and capillary stagnation during the terminal stage We have no direct

observational evidence concerning the circulation in the liver proper although several studies in this regard have been reported (4 5) Your calculations indicate certain discrepancies in over all splanchnic blood flow evidence for which cannot be provided in the bowel proper its mesenteric appendages and probably not in the liver

Remington Are you making a distinction between the circulation in the bowel and the mesentery?

Zweifach I am drawing a distinction between the capillary circulation in the various mesenteries proper and the flow through the large veins and arteries which enter and leave the bowel by way of the mesentery Observations of these large blood vessels should reflect accurately disturbances in blood flow through the intestines

Horvath Is the reduction here greater than that in any other area?

Zweifach With the onset of hypotension (65 mm Hg or less) following hemorrhage, the flow through the small blood vessels of the skin and skeletal muscle almost ceases This is accompanied by extensive vasoconstriction of the arteries arterioles, and veins in these tissues. At this stage, there is only a moderate narrowing of the arteries and veins in the mesentery with the slowing in blood flow roughly parallel to the degree of hypotension When the blood pressure falls below critical levels (in the anesthetized dog below from 35 to 40 mm Hg) marked vasoconstriction of the mesenteric arteries and veins develops with little or no unidirectional flow in the veins

Bradley You seem to be contradicting yourself First you seem to say that the blood flow falls in proportion to the decrease in blood pressure, and then you speak of vasoconstriction. Is that not contradictory?

Zweifach Why am I contradicting myself? I say when an animal is bled the blood pressure falls

Remington It would seem that the term *vasoconstriction* should be reserved for a greater diameter decrease than would accompany through passive recoil, the reduction in pressure

Zweifach I am using the term *vasoconstriction* to indicate a narrowing of the vessel under observation with no implication concerning the factors responsible for its narrowing

Fremont Smith Certainly the vessel has contracted to get a reduced diameter

Horvath The important part is how much blood is going through the vessel In my own observations using iodinated albumen and radioactive chrome, the distribution of circulating fluid is no different in an animal in hemorrhagic shock than in the normal state We find as a rule

that there is about 28 per cent of the total circulating blood volume in the splanchnic bed in both groups

Baez. May I ask at what time during the hemorrhagic experiment these measurements were taken?

Horvath About an hour after they have been maintained at an arterial blood pressure of 40 mm. Hg So it is really a true, severe state of shock. The difference between the volume of circulating blood and the volume of apparent disturbance in the flow, which can be determined by the visual observations may be great

Remington What do you mean by the volume of the circulating blood?

Horvath The volume of blood in the total circulation is reduced by a significant fraction by continued bleeding The volume removed is greater than that replaced for instance, by movement of fluid from the intravascular spaces

Baez. I believe the difficulty arises because we are speaking of two different things namely, arterial or arteriolar vasoconstriction versus blood flow which were recorded by different observers in separate experiments and most likely at different times Visible and measurable narrowing of the arteries and large arterioles occurs during hemorrhagic hypotension, as Dr Zweifach described, either as an active process caused by neurogenic or humoral influences or as a passive adjustment to a decrease in the head of pressure This adjustment, however would not necessarily be reflected in blood flow measurements made in the portal or hepatic veins A rapid and massive passage of interstitial fluid into the vascular compartment occurs when the hydrostatic head of pressure decreases and the muscular metarterioles and precapillary sphincters are actively closed. This could account at least in part, for the unchanged blood flow measured in the portal and hepatic veins

Dr Bradley did I understand you correctly that the blood flow in the splanchnic viscera increases again after a period of reduced flow following hemorrhage?

Bradley No I said the blood flow seemed to return toward normal, that is, toward the control levels

Baez. When does this occur?

Bradley Perhaps I can answer this question more clearly by presenting data collected in our laboratory by Dr Heinemann, Dr Smythe, and Dr Marks (1) In Figure 1 values for estimated hepatic blood flow (EHBF) measured by the bromsulphalein method and expressed in terms of the control values are plotted against time for eight dogs studied before and following blood loss amounting to approximately 2 per cent of the body weight Blood flow fell sharply after hemorrhage,

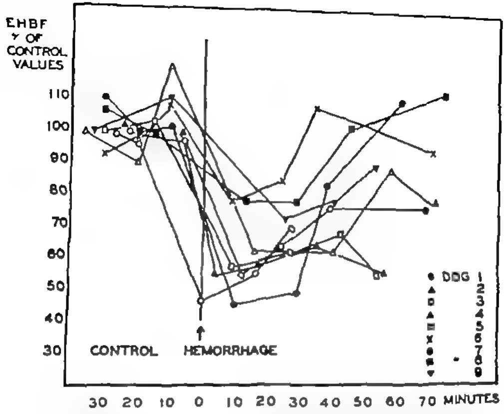

FIGURE 1 Effect of hemorrhage on estimated hepatic blood flow in dogs. Reprinted, by permission, from Heinemann, H. O., Smythe, C. M., and Marks, P. A. Effect of hemorrhage on estimated hepatic blood flow and renal blood flow in dogs. *Am J Physiol* 174 352 (1953)

and then returned within one hour to or toward the control level in the absence of restoration of blood pressure or cardiac output

Horvath There is no question that after a single massive hemorrhage, the estimated splanchnic blood flow does tend to return to normal I think there have been several other reports besides theirs that showed the same thing (6)

Zweifach Doesn t the same type of readjustment develop in most other areas of the body although possibly to a different degree?

Horvath This occurs, if I am not mistaken despite the fact the cardiac output is still low

Bradley Yes

Dobson How can this occur all over the body and the cardiac out put still remain low? If the cardiac output does stay low while the splanchnic circulation returns toward normal with time after hemor rhage, then some tissue or tissues elsewhere in the body must be getting less and less blood flow with time after the hemorrhage

Bradley That is true In the experiment shown in Figure 2, from a study by Reynell *et al* (3), it may be seen that the fall in blood pressure and cardiac output (measured by the direct Fick method) after hemor rhage persisted until the time when recovery in EHBF was evident. Thus, vasodilation apparently develops within the splanchnic bed, pre sumably accompanied by more pronounced vasoconstriction elsewhere in the body

Selkurt Because of the uncertainty of the bromsulphalein method, we made measurements with the bristle flow meter developed by Dr Brecher and Dr Praglin (7) in our department The meter is inserted directly into the portal vein of dogs For the hepatic artery flow flow measurement was made by a carotid hepatic artery shunt, because the flow meter head was too large to insert directly into the artery

We employed the hemorrhagic shock procedure previously used in

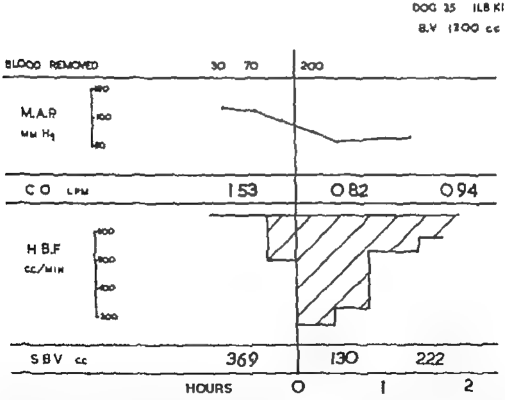

FIGURE 2 Changes in mean arterial pressure (M.A.P) cardiac output (C.O) estimated hepatic blood flow *(H.B F) and splanchnic blood* volume (S.B.V) after acute blood loss. The major hemorrhage was produced at time 0 the first 100 ml. of blood were removed for analytical purposes. Reprinted, by permission, from Reynell, P C. Marks, P A., Chidsey C. and Bradley S E. Changes in splanchnic blood volume and splanchnic blood flow in dogs after haemorrhage *Clin Sc 14, 407 (1955)*

our laboratory Figure 3 shows a representative experiment with arterial blood pressure at the top immediately below this the bleeding volume is given as per cent of body weight then the portal vein flow and hepatic artery flow are given next are the pressures in the portal vein and hepatic artery in mm Hg and at the bottom is a calculation of resistance in these vascular beds by the A V pressure difference over flow (P/F ratio)

Resistance increases in the hepatic artery immediately with hemorrhage but it subsides later It increases with further bleeding in the 40-mm. Hg period The mesenteric resistance does not appear to be significantly increased with initial hemorrhage but does so later when the animal is bled down to 40 mm. Hg arterial blood pressure.

An interesting thing about these experiments was that upon transfusion of blood there was in every instance a marked hyperemia of the portal vein flow as is evidenced by the very marked overshooting of

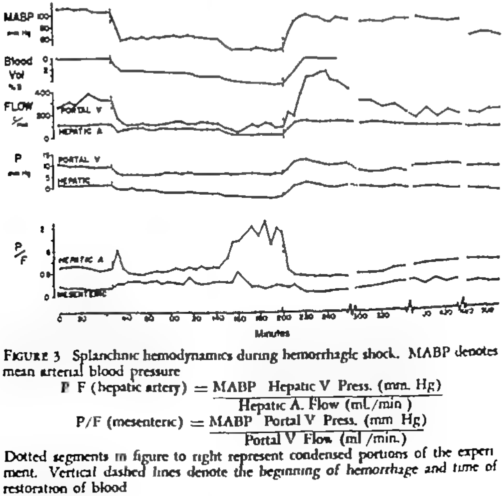

FIGURE 3 Splanchnic hemodynamics during hemorrhagic shock. MABP denotes mean arterial blood pressure

$$P/F\ (\text{hepatic artery}) = \frac{\text{MABP} \quad \text{Hepatic V Press. (mm. Hg)}}{\text{Hepatic A. Flow (ml./min.)}}$$

$$P/F\ (\text{mesenteric}) = \frac{\text{MABP} \quad \text{Portal V Press. (mm Hg)}}{\text{Portal V Flow (ml./min.)}}$$

Dotted segments in figure to right represent condensed portions of the experiment. Vertical dashed lines denote the beginning of hemorrhage and time of restoration of blood

flow The hyperemia is not so marked in the hepatic artery however The calculated resistance in the portal vein circuit (mesenteric) was always below control on transfusion, sometimes decreasing by one half

The next experiment shows the changes a bit more dramatically

Frank Were the animals anesthetized?

Selkurt With pentobarbital anesthesia They have been laparotomized, a procedure necessitated by insertion of flow meters (8)

Figure 4 shows another experiment with the same setup In this experiment there was a definite tendency for the portal vein flow to recover about 30 minutes after hemorrhage the hepatic artery did also but not to the same degree After hemorrhage, hepatic artery resistance declined continuously during the period of hypotension. Mesenteric resistance increased somewhat at first, then declined later in hypotension. On transfusion, there was a very marked hyperemia of the mesenteric bed, with tremendous overshooting of the portal vein flow The calculated resistance of the splanchnic bed was considerably reduced below control for some 2 hours posttransfusion. The hepatic artery flow did not participate so markedly in the hyperemic phenomenon

Thus in a group of dogs totaling about a dozen, we were surprised to find that there was not usually a marked increase of splanchnic vascular

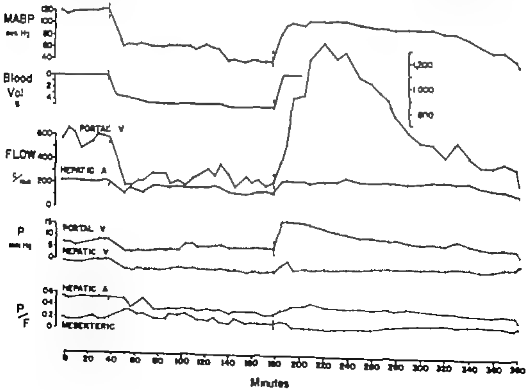

FIGURE 4 Representative experiment of more limited survival time.

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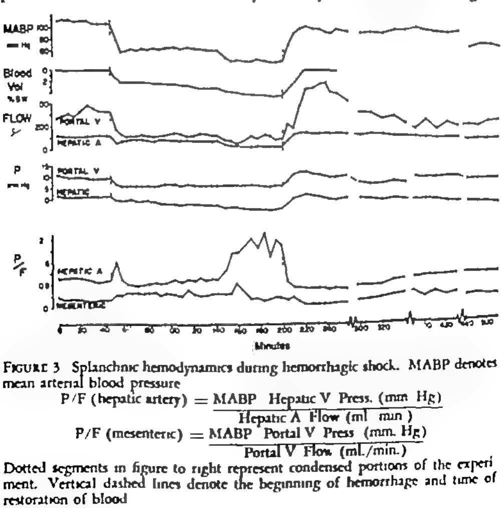

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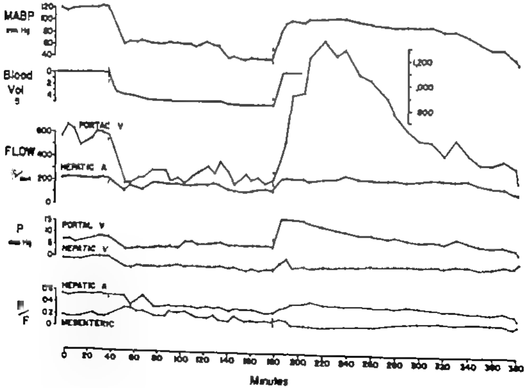

FIGURE 4. Representative experiment of more limited survival time

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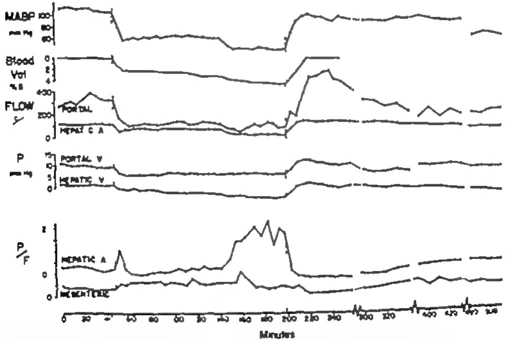

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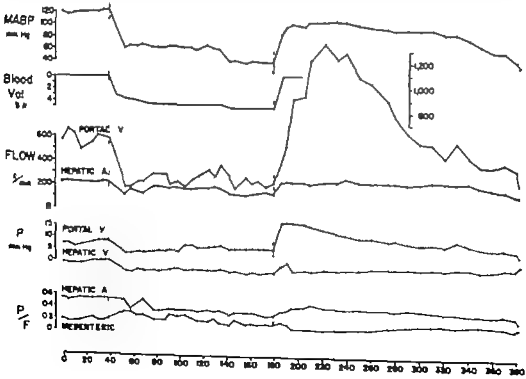

FIGURE 4 Representative experiment of more limited survival time.

resistance upon bleeding and when it did happen it was not maintained with prolonged hemorrhagic hypotension. Flow relative to pressure tended to increase somewhat, which, in a way supplements what Dr Bradley has said and opens the problem of what the mechanism is We just do not know why at present

DeBakey How do you account for the marked increase in portal vein flow with transfusion? Where does that blood come from?

Selkurt It appears as though the blood is being shunted preferentially into the portal vein Of course, we would like to go on at that point and suggest that this may be an indication of splanchnic pooling, and perhaps such a mechanism is made manifest in these data. There is an elevation of portal pressure. We presume that this is an indication of a wide open splanchnic bed The arterial pressure never in our experience, came back to control which indicated that the blood might be accumulating in some part of the vascular bed out of active circulation

Horvath When you are talking about the hepatic artery flow what blood vessel are you talking about?

Selkurt It is the common hepatic artery We did not ligate the end supplying the duodenum It is possible that the hyperemia in the intestinal bed complicates the flow and resistance interpretation in the hepatic artery because some blood goes to the intestine

Nickerson One difference between the data in Figure 4 and those in Figure 3 is that the hepatic artery resistance did not increase much during the second period of bleeding Was this difference correlated with duration of survival or anything else in the animal s behavior as compared to other experiments in which the hepatic artery resistance increased markedly?

Selkurt This is a point of considerable concern to us but I can offer no explanation. The first animal survived longer than the second suggesting an over all better vasomotor compensation than the second

Frank Dr Selkurt does this conflict with the suggestion of Wiggers and Opdyke (9) that resistance in mesenteric arteries is reduced rather than increased during hemorrhagic hypotension?

Selkurt Their work had more to do with hepatic resistance changes than mesenteric We are concerned here with hepatic artery flow It is a complicated matter to assess what happens to portal inflow into the liver Obviously direct information cannot be obtained with the approach of Wiggers and Opdyke They based their conclusions on the fact that the portal venous pressure increased relative to an estimated flow and said that this might be caused by increased hepatic resistance to portal inflow

It is unsafe to assume that hepatic artery flow is going to behave in a manner similar to portal inflow, and I should hesitate to make conclusions concerning changes in portal inflow resistance on the basis of the hepatic artery changes It is true in our experiments that, after an initial decrease following hemorrhage, the portal pressure increased during sustained hypotension and it increased noticeably over control after transfusion. Perhaps the rise in portal pressure during hypotension was a result of the development of increased resistance to portal inflow However it might have been a manifestation of development of hyperemia in the gut It is hard to say which or what combination of the two is the important factor

Knisely I missed one point of the description of measuring the flow through the hepatic artery Did you have to cut the artery completely across in order to attach the instruments to it?

Selkurt We cannulated the carotid and ran an external circuit through the bristle flow meter and inserted a cannula in the hepatic artery

Knisely You did not cut if off?

Selkurt The central end of the common hepatic artery was tied at its junction with the celiac, and it was perfused peripherally

Knisely I am especially curious because there are large vasomotor nerves along the hepatic artery Did you cut them or not?

Selkurt We were careful to dissect the nerves free and pass ligatures between the nerves and the vessel. This does not mean that nerve damage is not possible but we took care not to sever nerve tracts, presumably they are mainly intact

Frank Dr Shorr we have heard evidence of two sorts from Dr Bradley and Dr Selkurt bearing on hepatic blood flow Perhaps the group would like to hear something of the results of direct cannulation of a hepatic vein. We passed a large catheter through the jugular vein into a hepatic vein, did a brief laparotomy under ether anesthesia to tie it snugly in, and measured outflow rates (returning the blood to a jugular or femoral vein) when the dog was thoroughly awake. We found that when we bled the dog to an arterial pressure 30 mm. Hg, the hepatic venous outflow rate fell on the average to some 18 per cent of control values and as hours went by at that same low blood pressure the venous outflow tended to fall a bit further to about 13 per cent of control Our elevated reservoir system, of course, defeats compensatory mechanisms. The dog bleeds out any additional blood he is able to mobilize.

This preparation makes it possible for samples of the hepatic venous blood to be taken Of course, we are aware the hepatic blood

resistance upon bleeding and when it did happen it was not maintained with prolonged hemorrhagic hypotension. Flow relative to pressure tended to increase somewhat, which, in a way supplements what Dr Bradley has said and opens the problem of what the mechanism is We just do not know why at present

DeBakey How do you account for the marked increase in portal vein flow with transfusion? Where does that blood come from?

Selkurt It appears as though the blood is being shunted preferentially into the portal vein. Of course, we would like to go on at that point and suggest that this may be an indication of splanchnic pooling and perhaps such a mechanism is made manifest in these data. There is an elevation of portal pressure. We presume that this is an indication of a wide open splanchnic bed The arterial pressure never in our experience, came back to control which indicated that the blood might be accumulating in some part of the vascular bed out of active circulation

Horvath When you are talking about the hepatic artery flow what blood vessel are you talking about?

Selkurt It is the common hepatic artery We did not ligate the end supplying the duodenum It is possible that the hyperemia in the intestinal bed complicates the flow and resistance interpretation in the hepatic artery because some blood goes to the intestine

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becomes anoxic under these conditions It becomes very anoxic in the first hours of shock, the hepatic venous oxygen saturation falls to 5 per cent from its average control value of 56 per cent.

Horvath You talk about reduction of oxygen content That has no meaning as far as anoxia of the tissues is concerned What does the reduction of oxygen content mean?

Selkurt Shall we quote the heart?

Frank That is true that the normal coronary venous blood has a low oxygen content

Horvath You would say the cardiac tissue is anoxic?

Frank I would say not

Horvath The finding does not prove that the tissue is anoxic All it says is that the withdrawal of oxygen from the blood is greater

Frank Actually I gave only the oxygen saturation of blood leaving the liver whether the tissue ended up with as much oxygen as it needed I do not know As the hours passed the hepatic venous oxygen saturation went lower, to 3 per cent, and transfusion did not restore the normal value for saturation, or flow either in this experiment

Knisely Are there enough data to calculate the flow and oxygen concentration going in and coming out?

Engel Isn t it fair to say that on the basis of the data available the liver is hypoxic?

Shorr The liver is converted partially and sometimes completely to an anaerobic organ.

Selkurt You mean that the indication is that the liver does not become anoxic?

Horvath Yes I raised the question whether the liver does become anoxic The very fact that the liver takes more oxygen out of the blood perfusing it does not indicate that it becomes anoxic.

Selkurt The findings would so indicate if the flow were reduced—

Horvath Not necessarily It simply means that if the flow is reduced, the liver is taking out more oxygen per unit of blood flowing through it.

Engel The liver *in situ* in the shocked animal behaves metabolically the same as the liver taken out of a normal animal, sliced and incubated at a low oxygen tension. The data are reasonably good Certainly Dr Shorr has such data and we showed years ago that the liver is temporarily or even permanently behaving as if it has a limited oxygen supply hence I do not see the reason for belaboring the issue

Horvath The finding does not mean the liver cell is working at a different oxygen partial pressure it may be the very same as the control I do not think there is any evidence to say definitely

TABLE I

Splanchnic Blood Flow and O_2 Utilization During Prolonged Hemorrhagic Hypotension in the Dog
(means of nine experiments)

	Control	60 mm Hg	40 mm Hg
Blood flow ml/min			
Portal vein	309	142	99
Hepatic artery	177	123	70
O_2 content vol/per cent			
Arterial	18.1	15.8	17.0
Portal vein	11.6	5.6	5.6
Hepatic vein	9.3	3.3	2.1
A V O_2 difference vol/per cent			
Arterial portal vein	6.5	10.2	11.4
Portal vein Hepatic vein	2.3	2.3	3.5
Arterial Hepatic vein	8.8	12.5	14.9
O utilization ml/min*			
Intestinal	309 x 6.5 = 20.1	142 x 10.2 = 14.5	99 x 11.4 = 11.3
Liver			
Portal vein	309 x 2.3 = 7.11	142 x 2.3 = 3.26	99 x 3.5 = 3.46
Hepatic artery	177 x 8.8 = 15.53	123 x 12.5 = 15.40	70 x 14.9 = 10.42
Total	22.64	18.66	13.88
Total splanchnic	42.76	33.16	25.16

*In this table the O_2 utilization has been calculated from the average O_2 difference and the average blood flow hence the slight differences from the figures plotted in Figure 5 on page 23

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Horvath The finding does not mean the liver cell is working at a different oxygen partial pressure it may be the very same as the control. I do not think there is any evidence to say definitely

Selkurt Oxygen utilization determinations along with flow measurement are apropos at this time.

We did A V O_2 differences across the intestinal and liver beds combined with flow to see what happened to O_2 utilization. It appeared to decrease during prolonged hypotension

Table 1 shows the mean data during the control and the hypotension periods of nine experiments in which the animal survived the period of hypotension Figure 5 is a plot of these data The second and third curves from the top show the portal vein and hepatic artery flow then O_2 content of arterial portal vein and hepatic vein blood are shown during control, hypotension, and after transfusion. The lower two curves show O_2 utilization per minute by the liver and intestine Liver utilization dropped from about 25 ml per min to about 13 late in hypotension The intestinal bed O_2 utilization, which represented quite a bit of the total splanchnic O_2 utilization, also decreased by about 50 per cent during hypotension On transfusion, the liver utilization returned to control but for some reason the intestinal bed did not

Levine The reduction in O_2 utilization is about the same as the reduction in the portal blood flow which also seems to be 50 per cent

Selkurt Obviously the O_2 utilization per minute is going to be dependent on the supply by both the portal vein and the hepatic artery

Knisely Do these figures include the artery flow?

Selkurt They include the hepatic artery and portal vein flow The intestinal utilization is the arterial-portal vein O_2 difference times the portal flow The liver utilization is the sum of the portal vein hepatic vein O_2 difference times the portal flow plus the artery hepatic vein O_2 difference times the hepatic artery flow

Bradley Were those dogs splenectomized?

Selkurt No, they had an intact spleen

Bradley Do you include the blood flow from the spleen?

Selkurt The data included the spleen and intestinal flow into the portal vein.

Levine A question occurs to me at this point The oxygen consumption falls at most about 50 per cent The portal venous and hepatic artery flows decrease to about the same extent It would be expected therefore that the A V difference would remain the same yet the difference between the arterial and the hepatic vein O_2 contents across the whole splanchnic bed has widened and if the blood flow has decreased 50 per cent and the A V difference has increased then utilization cannot be just half of the control utilization

Selkurt You have perhaps confused the portal vein oxygen content with the portal vein hepatic vein difference which has not changed

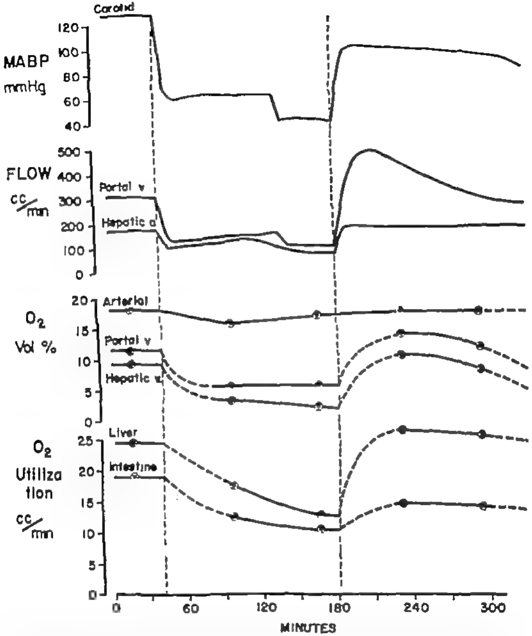

FIGURE 5 Average trends of nine experiments, showing the relationship of blood oxygen content and oxygen utilization to flow (See also legend to Figure 3.) In this figure the O_2 utilization is a plot of the average of the utilization calculated for each individual experiment.

appreciably in the first period of hypotension In the second period of hypotension the flows in both artery and vein have decreased so greatly that despite an increase in the O_2 uptake (O_2 difference) in both artery and vein, the O_2 utilization by the liver has decreased still further

Horvath Our own measurements indicate that the per cent of

utilization of oxygen by the liver or hepatic splanchnic bed is the same during both of those states

Selkurt The portal flow is about 310 ml during the control It decreases to about 130 ml /min on hemorrhage Then it declines to 100 ml /min on further bleeding to the 40-mm Hg level Hepatic artery flow decreases from 170 to 100 ml /min then to 70 ml /min during severe hypotension There is marked overshooting of portal flow on transfusion but hepatic artery flow tends to come back only to control The O_2 content during control is 18 11 5 and 9 5 vol /per cent, respectively for arterial portal vein, and hepatic vein blood

DeBakey When you say arterial blood what do you mean?

Selkurt Systemic arterial oxygen On bleeding the content drops slightly in the arterial blood Portal vein content decreases to about 6 vol./per cent and hepatic vein to about 4 vol /per cent decreasing further to about 2 vol /per cent late in hypotension

Next we multiply the A V differences of the O_2 contents by the blood flow to obtain utilization The method for obtaining intestinal utilization was previously discussed The liver utilization can be specifically computed by the portal hepatic vein difference multiplied by portal inflow This is one component supplying oxygen to the liver The hepatic artery O_2 supply was computed from the arterial hepatic vein O_2 difference times the hepatic artery flow

Burch If you were to guess which of the procedures would be erroneous, assuming there is an error would you consider the measurements of blood flow to be in error?

Selkurt I do not see how they could be These are by a direct method

Fremont Smith Which is the most vulnerable of your methods of measurement?

Selkurt I do not think either one could have much error Let us say on the outside the combination would be 10 per cent

Dobson It seems to me that everything adds up as it should

Selkurt It is confusing perhaps because the intestinal bed takes out considerable oxygen which to an extent deprives the liver

Fine Don t you have to take account of the oxygen removed by the liver from the portal vein as well as from the hepatic artery?

Selkurt Yes Actually as it turns out in these studies the liver got most of its oxygen from the hepatic artery mainly because the portal oxygen content was quite depleted after passing through the intestinal bed

Rappaport What I noted in this discussion was that the oxygen consumption of the extrahepatic tissue was not discussed There are some recent studies by Smith Roth and Grace (10) which show

that the arterial portal oxygen saturation gradient increases very much in hemorrhagic shock, and that in the period of severe hypotension the liver depends almost exclusively on the arterial blood supply If this factor is injected into the discussion, some of the difficulties in understanding Dr Selkurt s curves might disappear The portal blood brings in very little oxygen during the period of hypotension

Selkurt This is an important point I mentioned earlier In these studies during the hemorrhage the supply by way of the artery is about 80 per cent or more of the total. The A V difference must be looked upon almost entirely in terms of what the hepatic artery flow is doing

Rappaport I should like to make another point. I mention this because we know that in the normal rat liver there is a vicarious relationship between portal and arterial supply From these studies it is evident that this relationship this interplay, still persists in hemorrhagic shock

This interplay is also proved by microscopic observations of the hepatic circulation *in vivo* made by workers in England (11) who bled some rats and observed that the portal venules shut down and almost disappeared from the microscopic field while blood continued to flow through the arterioles Thus we have a microscopic proof that in a period of blood loss the liver is in a great part supplied by arterial blood.

Bradley I think we have made a start on the first question. What we have elicited so far seems to indicate there are indeed differing activities in the different parts of the splanchnic vasculature. The hepatic artery and its derivative arterioles do seem to behave somewhat differently from the mesenteric pancreatic arterioles but it is still very difficult to find out exactly how and in what way these change relative to one another at different points during the development of the hypotensive state.

Knisely I should like to make a brief summary of all the known anatomic pathways

EDITOR S NOTE Most of the following material was inserted by Dr Knisely during the editing of the conference material.

> When we are doing experiments* in normal and pathologic physiology in any one species of animal, each of us bases his work on his own mental model of the anatomy of the territory in which he is working One purpose of the following anatomic descriptions is to enlarge our working mental models, and to give references to the literature

*The work from our laboratory presented in the Josiah Macy Jr Conferences on Shock and Circulatory Homeostasis is supported by the Office of Naval Research, Contract No Nonr rer(01) and USPHS Grant No. H1683(C2)

which will make it possible to enlarge and increase the accuracy of our mental models.

Two major classes of vessels may be emphasized those which bring blood into the territory drained by the portal vein, and those which do or may carry blood from the portal vein bed into the vena cava or other systemic vessels.

The several vascular beds of each of a series of organs drain into the portal vein The veins of the gastrointestinal tract from the lower end of the esophagus or the cardia of the stomach, to the anus This includes the specific capillary beds of the cardia of the stomach, the stomach itself the pyloric antrum, pylorus, duodenum, jejunum, ileum, appendix and the large intestine including the descending colon, rectum, and anus.

The vascular beds of the various mesenteries of the gastrointestinal tract including the mesoappendix and the vascular apparatus of the *greater omentum, a large usually forgotten structure, also drains into* the portal vein bed. To this must be added the spleen and the pancreas

The various segments of the gastrointestinal tract usually consist of three separable parts the smooth muscle both circular and longitudinal layers the specific mucosal lining and various types of gastrointestinal glands such as for instance, the Brunner s glands of the duodenum in man. The capillary beds of the smooth muscle of the absorptive inner surface of the gastrointestinal tract and of the various glandular systems, are anatomically different in the arrangement of vessels Very little is known about the vasomotor responses of the various specific sets of these capillary networks. In 1910 I carefully delineated the principles underlying the distribution of capillary networks (12)

In man, there certainly are large changes in the volume of blood flowing through the total vasculature of the gastrointestinal tract during different physiologic states. For instance, following a large meal at a time when an individual is resting his skeletal muscles, there will be very little blood flow through the muscles, but the cardiac output is high and a *large proportion of that cardiac output* is directed through gastrointestinal portal and liver vessels (13) The specific details of which particular sets of vessels have the fastest flow or which are shut off under various conditions remain to be explained

Rudolph Spanner (14) described arteriovenous anastomoses across the roots of the villi of the gut For more details of the blood supply of parts of the gastrointestinal tract, see Mall (15) Noer (16) von Möllendorff (17 18) and Braus (19) Noer (16) compares vascular distributions in a series of different species

Obviously a great deal of specific investigation on the distribution of blood vessels and the physiologic reactions of blood vessels in the smooth muscle of the parts of the gut, the various glandular tissues and the absorptive surfaces is long overdue

The arteries which enter the mesentery of the intestine may be divided into two major categories (*a*) those which go all the way across the mesentery and supply various parts of the gastrointestinal tract as described above and (*b*) arteries which enter the mesentery

and supply specific parts of the mesentery itself such as the sheets of connective tissue, small lymph nodes and the small discrete clusters of fat cells which Professor Fritz Wassermann calls fat organs (20,21,22)

These three types of capillary beds in mesentery certainly have different vasomotor behavior one from the other For instance, when focusing a microscope into the mesentery of rhesus monkeys, one may find that nearly all the smaller vessels supplying fat tissue are tightly shut off and contain no blood while the capillary beds of the sheets of mesentery have a rather good blood flow The vascular bed of each structure of the mesentery and of the *omentum* now deserve specific anatomic, physiologic, and pharmacologic study

The veins of the pancreas, which are of two separate classes drain into portal vein territory The classes are (*a*) the veins from the pancreatic islet tissue and (*b*) those from the acinar tissue. Each pancreatic islet (23 24) has its own blood supply quite discrete, perhaps as isolated as the blood supply of a kidney glomerulus

Anatomists are not agreed upon the distribution of small blood vessels within the spleen, and I myself am a protagonist in the major controversy consequently whatever I say about the spleen must be recognized as a statement by a protagonist

During normal physiology when the blood cells are not agglutinated, the spleen stores concentrated red blood cells This it does by means of precisely controlled sphincter mechanisms located at various points on or as a part of the splenic vascular apparatus. For drawings showing this splenic vascular apparatus, see Cowdry (25) and Bailey (26)

For instance, a sphincter at the downstream end of a splenic sinusoid contracts tightly shut, blood continues to be delivered to the upstream end of the sinusoid, the fluid part of the blood passes out through the sinusoid wall, and the sinusoid becomes filled with and stores concentrated red cells For details of splenic vascular architecture and physiology under strictly healthy and uninjured conditions, see Knisely (27) Peck and Hoerr (28 29) Palm (30) and Nakata (31) For a detailed analysis of our current ignorance, see Knisely (32)

The exact details of the vascular apparatus of the whole liver and of the liver lobule are now receiving the attention of investigators. See, for instance, Healey and Schroy (33) Healey Schroy and Sorensen (34) and Healey (35) There is more knowledge of the vascular architecture of the liver lobule for the frog than for any other species For this see Knisely Bloch, and Warner (36) Many details of the vascular architecture of the liver lobule of the rhesus monkey are similar to corresponding details for the frog. For a diagram of the frog liver lobule, see Cowdry (25) and Bloch (37)

The distribution of the hepatic arteries to and within the hepatic tissue is being investigated intensively Soskin, Essex, Herrick, and Mann (38) found that in dogs sometimes as much as 90 per cent of the blood entering the liver came in by way of the hepatic arteries.

Markowitz and colleagues (39-45) have found that in the dog a supply of highly oxygenated arterial blood by way of the hepatic

arteries is necessary in order to prevent the multiplication of anaerobic bacteria in the liver Obviously this may be highly important in shock in the dog, and perhaps in other species

The exact distribution of the hepatic arteries to and within the liver lobule is not yet known for a wide variety of species. The concept that the hepatic artery does not supply the sinusoids of the liver lobule, which was put forth by Aunap (46) and based upon the failure of injected materials to reach the lobule, certainly is erroneous. In our own laboratory terminal hepatic arterioles delivering bright red highly oxygenated arterial blood into the sinusoids have been seen in rhesus monkeys, rats, frogs, and several other species.

For details of the literature on the vascular supply of the liver see Lichtman (47 48 49) all the Josiah Macy Jr Conferences on Liver Injury (50-54) and the publications of Rappaport (55 56)

Michels (57) should be consulted in detail for the distribution of arteries to all parts of the upper abdominal organs. His book provides much detailed knowledge, including variations within species and a tremendous, carefully selected bibliography See also von Bardeleben (58)

When we are doing experiments in gross physiology and gross pathologic physiology experiments in which blood pressure is determined in large vessels and flow measured in other large vessels, we often lose sight of the fact that there are precise anatomic locations in which highly contractile, specifically controlled sphincters exist. Among these are the small arteries and terminal arterioles of each and every organ named above arteriovenous anastomoses such as those across the villus described by Spanner (14) sphincter mechanisms at various points on the splenic vasculature (27,28 29 and 59) specific contractile mechanisms in the liver in the frog, the terminal hepatic artery supplying parts of lobules, the arterioportal anastomoses (which when open pour arterial blood through the tips of the portal vein into the hepatic sinusoids) and a sphincter located where each sinusoid accepts blood from a portal vein tip, and another where each sinusoid joins a central vein.

Three specific classes of sphincter are known which can control the flow of blood from the liver into or through the hepatic veins (*a*) in the frog and rhesus monkey sphincters where the sinusoid joins the central vein of the lobule (*b*) in several species, Deysach s (60) small sluice channel which controls the flow of blood from a group of sinusoids into a large central vein or small sublobular vein of the liver and (*c*) contractile mechanisms of the hepatic veins themselves. The latter in the dog, can contract tightly shut and directly cause the death of the animal (61)

The above material outlines briefly pathways whereby blood can enter the portal vein bed and the control of the flow of blood through the hepatic veins into the inferior vena cava.

The following material outlines what is known about collateral branches between the portal vein bed and the systemic veins, the so-called portacaval anastomoses."

In the literature, connections between the portal vein and the vena cava are ordinarily called portacaval anastomoses. Many of such blood pathways are not directly between the portal vein and the vena cava, but rather join namable branches of the portal vein with namable branches of systemic veins. A better term, thus, is portasystemic anastomoses.

Many people working in the pathologic physiology of shock assume that *all* the blood which enters the portal vein bed passes to the vena cava through the hepatic veins. Many measurements of the flow of blood through hepatic veins are interpreted as though the investigator had been measuring the total outflow from the portal vein bed

Quite a number of collateral connections exist between parts of the portal vein bed and branches of the inferior vena cava in every species thus far studied, including man.

Dr Ralph Comer a member of the Department of Anatomy of the Medical College of South Carolina at Charleston, has prepared for us a list of portasystemic anastomoses as follows

'Branches of the systemic circulation which routinely communicate with branches of the portal circulation are esophageal, hemorrhoidal, epigastric, phrenic, lumbar renal, spermatic, ovarian, uterine and vesicle veins. Large connections between major portal tributaries and the inferior vena cava have been described in man but are generally considered anomalous.

Portacaval anastomoses exist where the (*a*) columnar gut epithelium joins with squamous epithelium (*b*) every place where portions of the gut have become retroperitoneal developmentally and (*c*) at sites where foetal circulation has failed to obliterate. The connections were recognized and well described by 1900 Charpy writing on the portal vein in Poirier's *Traité d'Anatomie Humaine* (62) devoted a section to each area in which portacaval anastomoses had been described The richness of the literature and the extent of the knowledge of the anatomy of the portacaval connections can be estimated from the following list of sites taken from Charpy's paper (62) and their investigators

1 Squamo-columnar junctions
   a. Esophageal.
   b Rectal
2 Foetal Circulation.

Interest in portacaval connections has not decreased Recent papers on this subject have been published by Wermuth (63) Spanner (64) Thamm (65) Edwards (66) and Holmes and Lovitt (67) These investigators did not add a great deal to our knowledge of where connections exist but their reports are of interest because each mention *size* of the connections or *frequency* of connections in the areas they investigated

How frequently are there macroscopic communications? Spanner (64) says that in the area where the branches of the inferior mesenteric vein overlies the left spermatic vein there were at least four connections between these veins in all cadavers studied Wermuth (63) investigating connections between uterine vein and hemorrhoidal veins describes

four cases out of fourteen in detail. In these four cases communications at least 1 mm in diameter existed. Holmes and Lovitt (67) saw periesophageal connections of 1 to 2 mm in diameter in the specimens they studied. Edwards (66) said connections were not visible until filled with injection mass.

Species differences are not only possible but probable. Krakower (68), writing on schistosomiasis in the guinea pig, observes that in animals with a complete dorsal mesentery, e.g. without retroperitoneal duodenum and colon, portacaval anastomoses exist in smaller numbers but in larger sizes than in an animal like man where large portions of the area drained by portal circulation are fused with the dorsal body wall.

Some known species reactions to acute portal obstruction are summarized by Spellberg (69), p. 342. "For example, Child *et al.* (70) had 19 of 25 rhesus monkeys survive ligation of the portal vein. The six deaths were ascribed to causes other than the venous occlusion."

It is surprising to find that at least some rhesus monkeys can survive ligation of the portal vein. This points out the possible importance of portasystemic anastomoses *in health* as well as during various pathologic states when they may be widely dilated or may have developed into wide channels.

For each of a number of species, we need to know the numbers and the diameters of these anastomoses as they exist during health. We need to know the numbers and diameters during health as separate from the numbers and diameters which may exist as a result of disease. At present, some of the reports in the literature cannot be classified safely either as a part of the healthy anatomy or as a part of the response to a pathologic condition. The exact medical history of the animal or patient from whom the specimen was taken is not adequate to permit this important distinction. Often the authors of papers describing the anastomoses have not known, for each specimen, whether the findings were a part of healthy animals or were a result of a pathologic condition.

Because, in the minds of many, there is the fixed idea that all outflow from the portal vein bed necessarily passes through hepatic veins, anatomists who find collaterals between portal vein bed and systemic veins often list these as "anomalous." Thus far we have found no study sufficiently broad to make it possible to state with assurance that such portasystemic anastomoses actually are "anomalous."

Whether portasystemic anastomoses are under vasomotor control is not generally known. If there are specific investigations describing the anatomy and physiology of vasomotor control of such anastomoses, we have not thus far been able to find them.

Similarly, the possible hormonal chemical control of portasystemic anastomoses is not generally known for any species.

When attempting to learn the distribution of portasystemic vessels, one must keep in mind the differences between (*a*) the descriptions of structures which are in books and which must represent some set of average conditions, (*b*) a description of a single specimen every part of

which is precisely described, and (*c*) the embryologic or anatomic variations which one can expect to find regularly when examining a series of specimens all of the same general kind Possible species differences obviously must be kept in mind.

When studying through the above material, Dr Comer and I came to the conclusion that nothing less than a major co-ordinated attack on the anatomy physiology pharmacology and pathology of portasystemic anastomoses in each of a series of species can give us the real knowledge so seriously needed

In any case, no one can safely assume today that the total outflow from the portal vein bed is by way of the hepatic veins alone

Bradley The anatomic complexities noted by Dr Knisely make for hemodynamic complexity First, there is a group of arteriolar resistances in parallel between the aorta and the portal vein Another parallel set of resistances lies between the hepatic arterioles and the sinusoids Connecting the two terminuses of these circuits the portal vein and sinusoids is another resistance in the portal venules The sinusoids are, in turn connected with the inferior vena cava through the postsinusoidal resistance in the radicles of the hepatic veins and the portal vein may be linked directly with the inferior vena cava by collaterals which also impose a resistance to flow In its totality, this vascular net resembles that of the Wheatstone bridge. Formulation of the relationships between flows and resistances is extremely complex, requiring several simultaneous equations

Frank In looking over our data on hepatic venous samples it seemed that while the hematocrits of the portal blood and femoral blood were quite the same, the hematocrit of hepatic venous blood drawn simultaneously was fairly consistently 1 or 1½ points higher Is this something known to the ancient physiologists? Is it true? Is it agreed upon by those in this room?

Bradley In our experience the hematocrit of hepatic venous blood does not differ significantly from that of arterial blood obtained simultaneously

Frank Our large vessel hematocrits have been the same except in this one area. This amounts to fifteen or twenty observations It did seem as if the liver were taking plasma out of the blood in the form of lymph and concentrating red cells The action of the liver in transferring saline solution from circulating plasma to liver lymph and the acceleration of this action by epinephrine was pointed out by Lamson and Roca in 1921 (71)

Shorr Earlier in the discussion Dr Bradley asked some particularly relevant questions about liver blood flow and amongst them, why there is a spontaneous recovery of splanchnic blood flow under certain

circumstances of bleeding and why this spontaneous recovery doesn t take place under other conditions

Dr Knisely as a functional anatomist, provided us with a way of thinking which is going to be necessary in which all the discrete components channels, and processes involved in the splanchnic circula tion were cited and the complications emphasized which might be introduced discretely in each

Then Dr Selkurt provided evidence which seemed as if it were going to solve the problem, and this called forth a number of responses which challenged the data This was followed by a sug gestion of Dr Horvath s that there was no evidence at all that the liver was hypoxic in shock.

This brought to my mind the necessity for studying another variable If a liver becomes hypoxic it is going to shift over to an hypoxic and anaerobic type of metabolism. Another parameter must be added therefore to the ones that have already been cited namely the metabolic pattern of the liver We must use the evidences for the shift to an anaerobic type of metabolism as an indication that the liver blood flow is beginning to be reduced to such an extent that it now jeopardizes the facultative aerobic type of metabolism under which the liver exists

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THE BACTERIAL FACTOR IN EXPERIMENTAL SHOCK

JACOB FINE
Department of Surgery
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Boston, Mass

DR PILLEMER IS NOW INVOLVED in our work, and later in the discussion he will present a general review of the properdin problem We can then examine our data on the bactericidal mechanisms in shock in the light of his observations.

The curve shown in Figure 6 describes the standard experiment we employ for the study of hemorrhagic shock The animal is not medicated except for morphine given several hours in advance of bleeding The femoral artery bleeds into an elevated reservoir until the blood pressure reaches 30 mm. Hg and remains there. Eventually blood runs back from the reservoir to the artery

If after 2 hours, we shut off the femoral artery and return all the blood still in the reservoir to the femoral vein, the pressor response is prompt and adequate and the recovery rate is about 90 per cent. By recovery rate I mean sustained survival and not merely for 24 or 48 hours If instead, we do not transfuse until about half of the total shed volume has returned to the animal, the pressor response is transient and death occurs soon thereafter in an average of 6 hours after the bleeding was started

The problem is to decide why the animal exposed for much longer than 2 hours eventually becomes refractory to the transfusion. The data presented here will demonstrate that irreversibility to transfusion results from collapse of the bactericidal mechanisms and the subsequent liberation of bacterial toxins which paralyze the peripheral circulation

The first evidence in support of this view was the observation that antibiotics, given orally in advance of producing shock, enabled the dog to tolerate the shock state for much longer and to recover if a transfusion was given. Three different antibiotics produce such a result.

Shorr Would you read the figures?

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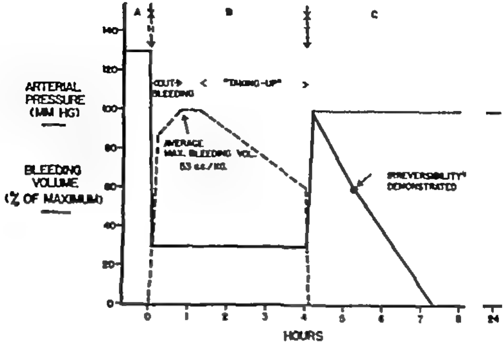

FIGURE 6. Standard experiment employed in the study of hemorrhagic shock. H = hemorrhage. T = transfusion of the blood remaining in the reservoir after the period of "taking up." Reprinted, by permission, from Frank, H. A., Fine, J., Friedman, E., Glotzer, P., Jacob, S., and Schwartz, A.: *Adrenal Cortical Therapy and Hepatic Vascular Resistance. Two Aspects of an Inquiry into the Pathologic Physiology of Experimental Hemorrhagic Shock.* Surgical Forum, American College of Surgeons, Philadelphia, W. B. Saunders Co., 1951 (p. 522).

Fine: Of 185 animals receiving no drug in advance, 14 per cent survived, whereas about 80 per cent survived if given antibiotics in advance.

Table II gives the original data. However, an important reservation must be made regarding these data, i.e., that they cannot be obtained today in our laboratory without observing special conditions that did not prevail when these experiments were first done. One of the most significant points I should like to make later in the discussion relates to this, and it involves Dr. Pillemer's discovery. We will return to it after his presentation.

Nickerson: Could you break these data down into 36-hour and permanent survivals as you did 4 years ago?

Fine: Yes. Of these, something like 10 per cent survived only 36 hours. Those that survived 48 hours did so indefinitely.

Shorr: What causes death in those that survive 36 hours?

Fine: They just peter out; however, survival for only 36 hours involves

a very significant issue I will come back to both of these subjects later

Obviously we are attempting to find bacteria in the animal to support our thesis The dog has *Clostridia* in all tissues except the central nervous system. From 191 cultures of blood tal en during all stages of shock we did not isolate any bacteria except an occasional *Clostridium* or *Pseudomonas* (Table III)

But cultures of blood, liver spleen etc., taken immediately after killing the animal or immediately after death yielded (Table IV) in addition to *Clostridia* a 25 per cent incidence of other intestinal flora, *Pseudomonas, Escherichia coli, Enterococci,* and *B proteus* What does this mean? I will only suggest that these bacteria did not get in after death, but were there before death and that the bactericidal mechanisms are of sufficient potency until the time of death to prevent the disclosure of their presence by the conventional cultural techniques

Kniseley Dr Fine, from which tissues did you get cultures?

Fine These are cultures of liver spleen, and peritoneum and of portal and vena cava blood I should point out that the number of positive cultures is higher in the portal than in the vena cava blood

Shorr What was the population count? Were these just positive cultures or did you make a count?

Fine These are positive cultures of varying richness We did not do counts Obviously, the state of shock results in tissue damage and presumably the amount of damage suffered after 5 hours of shock is greater than the amount suffered after 2 hours of shock Some idea of the extent of tissue damage even after a 2 hour exposure can be gained from Figure 7 which shows what happens when an animal is depleted of circulating prothrombin and how fast the blood regains its normal titer It is evident that in a normal animal the titer begins to rise within 60 hours and reaches a normal value in about 90 hours

But when an animal so depleted is exposed to shock for only one hour it will take an additional 100 hours approximately to restore the titer to normal. This is one way of measuring the degree of damage to tissue enzymes by the shock state

Green What are your co-ordinates?

Fine The abscissa is time in hours and the co-ordinate is titer of prothrombin in the blood. Presumably this is not just a special case, but is a representative example of injury to tissue enzymes

Fremont Smith This is a case of liver enzymes, isn t it?

Fine This is a case of enzymes in liver but I will risk making the inference that it is representative of damage to tissue enzymes in general

TABLE III

Incidence of Aerobic and Anaerobic Bacteria in 191 Cultures of Blood and Lymph During Hemorrhagic Shock

Source of Specimen	Before Shock	During Shock: Before transfusion (hr)				During Shock: After transfusion (hr)		
		1 to 2	2 to 4	4 to 6	6 to 8	¼	1 to	2 to 4
Group 1 10 dogs								
Portal vein	0	0	1	0	0	1	0	0
Vena cava	0	0	0	0	0	0	0	0
Aorta	0	0	0	0	0	1*	0	0
Group 2 3 dogs								
Portal vein	0	0	0	0		0	0	0
Vena cava	0	0	0	0		0	0	0
Aorta	0	0	0	0		0	0	0
Group 3 2 dogs								
Portal vein	0			2				
Aorta	0			0				
Group 4 3 dogs								
Portal vein	0							0
Aorta	0							0
Group 5 3 dogs								
Thoracic duct	0	0	0	0		0	0	0

**Pseudomonas*; the other four positive cultures showed *Cl.* [illegible] *Diphtheroids*, *subtilis*, *sarcinae* or nonhemolytic *Staphylococcus albus* were found in twelve cultures

Group 1 Blood samples drawn simultaneously from the three vessels at frequent intervals.

Group 2 Blood (1 to 2 ml) taken every min throughout the experiment and simultaneously replaced by equal volume of sterile blood

Groups 3 and 4 Samples taken from entire bleeding volume obtained by exsanguination via portal vein and aortic catheters

Group 5 Lymph before shock pooled and cultured Entire flow before transfusion pooled and cultured Entire flow after transfusion pooled and cultured

Reprinted by permission, from Jacob S Weizel H Gordon, E Korman H Schweinburg, F Frank, H and Fine J Bacterial action in development of irreversibility to transfusion in hemorrhagic shock in the dog *Am J Physiol 179 523 (1954)*

TABLE IV

Cultures of Blood Peritoneum, and Liver Taken Immediately After Death in Normal and Shocked Dogs

Group	No of Dogs in Group	Positive Cultures (Per cent of Group)							
		Portal Blood		Caval Blood		Peritoneum		Liver	
		Aerobic*	An aerobic	Aerobic*	An-aerobic	Aerobic*	An-aerobic	Aerobic*	An-aerobic
No shock (killed)	20	10	25	10	0	0	16	5	100
Shock (no antibiotic therapy)									
Survivors†	18	11	22	0	17	22	17	39	89
Nonsurvivors	43	23	30	14	33	14	37	60	95
Shock (antibiotic therapy)									
Survivors†	123	7	24	4	16	11	35	22	78
Nonsurvivors	111	24	41	15	35	21	39	37	94

*In 353 positive aerobic cultures (200 single 153 mixed) aerobic organisms were found in the following proportions *Pseudomonas* 41 per cent *B proteus* 22 per cent *E. coli* 19 per cent *Enterococci* 18 per cent.

†Survivors killed in good health 48 = 72 hr after shock experiment. Seventy-five contaminants (*diphtheroid subtilis* nonhemolytic *Staphylococcus albus*) were found in 1100 cultures.

Reprinted, by permission, from Jacob S., Weizel, H., Gordon, E. Korman, H., Schweinburg, F., Frank, H. and Fine, J Bacterial action in development of irreversibility to transfusion in hemorrhagic shock in the dog. *Am J Physiol* 179 523 (1954)

Fremont Smith Is there any evidence of enzyme damage outside the liver?

Fine Yes there is evidence of enzyme injury elsewhere I shall discuss that later

Haist Dr Fine, how were the prothrombin estimations done? Were they Quick prothrombin times?

Fine These prothrombins were done under the meticulous super

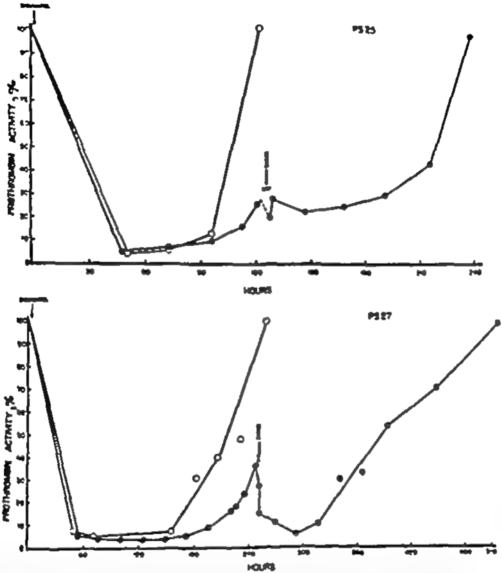

FIGURE 7 Delaying effect of a brief period of reversible hemorrhagic shock upon return of plasma prothrombin activity following preliminary reduction by dicumarol In each experiment (PS 25 and PS 27) both *curves* represent determinations in the same dog, after two separate administrations of the same dose of dicumarol (5 mg /kg. intramuscularly) In PS 25 the control curve of decline and recovery of prothrombin activity (O) was obtained a week before that (●) in which the recovery phase was interrupted by a period of shock (↓) In PS 27 the control curve (O) was obtained after the animal had recovered from the shock test (●) Each prothrombin activity value (dot or circle) was obtained by serial dilutions in parallel using normal or shock prothrombin free plasma There was no reduction of prothrombin conversion factor activity after dicumarol and only slight reduction after the period of hemorrhagic hypotension Reprinted by permission, from Frank, E. D Frank, H A and Fine J Traumatic shock XVIII Plasma prothrombin activity in hemorrhagic shock in the dog *Am J Phys* 167 199 (1951)

vision of Dr Benjamin Alexander * our hematologist I believe we used the two prevailing methods

Another example of a tissue enzyme that fails is ATP This was observed by Potter and his colleagues (1 2) during the early forties ATP drops abruptly during shock After 3 hours of shock there is a drop from 32 to about 8 units But after transfusion at this time it may or may not return to normal. Those dogs that recover show a nearly normal titer within 6 hours Those that do not respond to transfusion show a very much lower level (Figure 8)

I shall refer to injury of enzymes in other areas later on. At the moment, I should like to consider the damage to tissues involved in the destruction of bacteria. The normal dog will destroy bacteria given intravenously We tried three different species with survival in practically every instance. Table V shows that not over ten bacteria per ml. of blood are present 6 hours after injection At 24 hours there are in most cases none in the blood and none in the tissues On the other hand, an animal exposed to only 2 hours of shock and challenged likewise is in a very bad way All blood cultures are positive after 24 hours and remain positive until death, which occurs in every case within 1 to 4 days (Table VI) Table VII shows that the 2-hour shock animal as a rule clears the blood during the first 6 hours about as well as the normal animal

But in the animal that is allowed to go on and become irreversible to transfusion there is much more serious damage because in the blood of many such animals there is rapid multiplication of bacteria 6 hours after the bacteria are injected Some of the bloods show ten bacteria or less per ml some have perceptibly more but 40 per cent have very large numbers per milliliter

Zweifach Did you carry out white cell counts on dogs subjected to hypotension and blood replacement?

Fine We have done white cell counts occasionally The granulocyte count and the total count fell the former much more than the latter Transfusion after 2 hours is followed by the return of both counts to normal Transfusion after 4 to 5 hours does not change the counts We took the view that we could not incriminate the *Clostridia* for the reason that neomycin, which is as good as any other antibiotic, does not act upon *Clostridia* We, therefore, assumed that some other species is probably involved But we could not isolate any so we tried to demonstrate a bacterial factor in the tissues as follows Normal dogs and dogs

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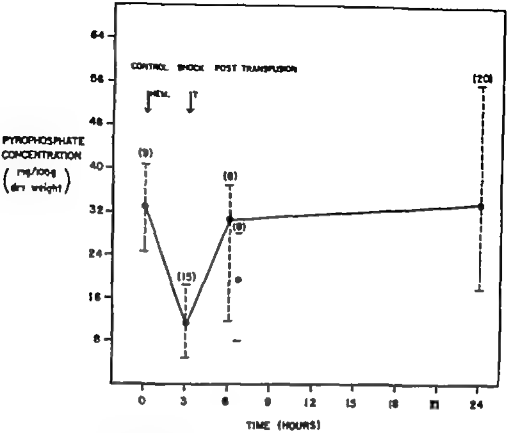

FIGURE 8 High-energy phosphate compounds in liver tissue of dog
Dashed bars = ranges of values
Dots = means
( ) = number of animals in group
Solid line connects means of survivors
Isolated dot and bars (after 6 hours) are values for nonsurvivors
Hem. = hemorrhage to arterial pressure 30 mm. Hg
Tx = replacement transfusion, 3 hours later

dying of *hemorrhagic shock in spite of transfusion* were killed and the livers removed immediately with sterile precautions They were promptly put in a deep freeze Later we prepared a mash from 200 gm of each liver using sterile precautions 150 ml of a suspension of each mash were *injected into the peritoneal cavity of a normal dog* or of a dog that had been exposed to shock for 2 hours In the latter the suspension was injected 1 hour before transfusion Seventy per cent of normal recipients survived this treatment whether the mash came from a normal or from a shocked dog s liver

Of the 2 hour shock dogs which received normal liver mash 90 per

TABLE V

Blood Clearance and Eventual Disposition of Bacteria Injected Into Normal Dogs

Exper No.	No. of Bacteria Injected (billion)	No. of Bact./ml Blood		Results
		At Beginning (million)	At 6 hr	
	E. coli			
1	10	9	10	Survived
2	44	2	Sterile*	Survived
3	12	9	Sterile*	Survived
4	28	13	Sterile*	Survived
5	84	56	Sterile*	Survived
6	10	6	Sterile*	Survived
7	50	33	Sterile*	Survived
8	9	4	Sterile*	Survived
9	13	65	Sterile*	Survived
10	21	10	2	Survived. Killed 3rd day. Blood, liver and spleen sterile
	Staphylococcus aureus			
11	10	4	Sterile	Survived
12	7	4	Sterile	Survived
13	3	13	Sterile	Survived
14	43	31	20	Survived. Killed 24 hr. Culture of blood, liver and spleen +
15	75	52	Sterile	Survived. Killed 96 hr. Blood sterile at 24, 48 and 96 hr. Liver and spleen sterile

*Sterile at 6 hours signifies no growth on plate cultures. But broth cultures were positive, signifying less than 10 bacteria/ml of blood. Cultures taken 4 hours or later designated "sterile" were sterile in broth as well as on plates.

TABLE V (Contd.)

Exper. No.	No. of Bacteria Injected (billion)	No. of Bact./ml. Blood: At beginning (million)	No. of Bact./ml. Blood: At 6 hr	Results
16	24	12	Sterile	Survived. Killed 5th day. Blood, liver and spleen sterile
	Clostridia†			
17	?	?	Few**	Survived. Killed 48 hr. Blood culture 24 hr —. Blood culture 48 hr +
18	?	?	Sterile	Survived. Killed 48 hr. Blood culture 24 hr +. Blood culture 48 hr —
19	?	?	Sterile	Survived. Killed 96 hr. Blood culture 24 hr +. Blood culture 48 hr —. Blood culture 72 hr —. Blood culture 94 hr —
20	?	?	Sterile	Survived. Killed 72 hr. Blood culture 24 hr —. Blood culture 48 hr —. Blood culture 72 hr —
21	?	?	Sterile	Survived. Killed 96 hr. Blood culture 24 hr —. Blood culture 48 hr —. Blood culture 72 hr —. Blood culture 96 hr —

†Heavy 24-hour growth in thioglycollate of a toxigenic strain (obtained from Dr. Milan Logan, Prof. of Biochemistry at the University of Cincinnati Medical School, Cincinnati, Ohio) which will not grow on the usual media except blood agar. Counts cannot be made because the deep colonies in a blood agar plate cannot be seen. The cloudiness of the thioglycollate culture was adjusted to approximate that of an *E. coli* standard so that some billions of *Clostridia* were injected.

**Gas production in thioglycollate was very slight and direct smear showed a few bacteria per oil immersion field.

Reprinted, by permission, from Schweinburg, F. B., Frank, H. A., and Fine, J.: Bacterial factor in experimental [illegible] Evidence for development of a bacterial factor which [illegible] for [illegible] transfusion and for the loss of the normal cap[illegible] *Physiol.* 179: 532 (1954)

TABLE VI

Blood Clearance and Eventual Disposition of Bacteria Injected Into Dogs in Hemorrhagic Shock 1 Hour After Shock Was Induced, Transfused After 2 Hours in Shock

Exper No.	No. of Bacteria Injected (billion)	No. of Bacteria/ml. of Blood: At Beginning (million)	No. of Bacteria/ml. of Blood: At 6 hr	Cultural Data: Blood	Cultural Data: Spleen	Cultural Data: Liver	Surv. Time, hr†
	Staphylococcus aureus						
1	15	10	200	24 hr +	+	+	24
2	15	10	20	24 hr +	+	+	24
3	45	30	6	18 hr +	+	+	18
4	45	26	4	24 hr +			
				48 hr +			
				72 hr +	+	+	72
5	35	24	40	24 hr +			
				48 hr +			
				72 hr +			
				96 hr +	+	+	96
6	35	17	4	24 hr +			
				72 hr +	+	+	72
7	75	50	20	+	+	+	24
	E. Coli						
8	19	8	20	24 hr +			
				48 hr +	+	+	48**
9	19	8	Sterile	24 hr –			
				48 hr +	+	+	48
10	12	8	5000	+	+	+	24**
11	12	8	30	+	+	+	18
12	17	8	2	24 hr +			
				48 hr +	+	+	48
13	17	8	8	24 hr +			
				48 hr +	+	+	48
	Clostridia						
14	?	?	+				6
15	?	?	+	24 hr +			
				48 hr +	+	+	48**
16	?	?	+	24 hr +			
				48 hr +	+	+	48**
17	?	?	+	24 hr +	+	+	24
18	?	?	+	24 hr +			
				48 hr +	+	+	48

Cultures of spleen and liver taken at death or as soon as possible thereafter
†The figures in this column are only approximate. Death occurred at or some hours preceding the time given
Killed when obviously very ill

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TABLE V (Contd.)

Exper No	No. of Bacteria Injected (billion)	No. of Bact./ml. Blood: At beginning (million)	No. of Bact./ml. Blood: At 6 hr	Results
16	24	12	Sterile	Survived. Killed 5th day. Blood, liver and spleen sterile
	Clostridia†			
17	?	?	Few**	Survived. Killed 48 hr. Blood culture 24 hr —. Blood culture 48 hr +
18	?	?	Sterile	Survived. Killed 48 hr. Blood culture 24 hr +. Blood culture 48 hr —
19	?	?	Sterile	Survived. Killed 96 hr. Blood culture 24 hr +. Blood culture 48 hr —. Blood culture 72 hr —. Blood culture 94 hr —
20	?	?	Sterile	Survived. Killed 72 hr. Blood culture 24 hr —. Blood culture 48 hr —. Blood culture 72 hr —
21	?	?	Sterile	Survived. Killed 96 hr. Blood culture 24 hr —. Blood culture 48 hr —. Blood culture 72 hr —. Blood culture 96 hr —

†Heavy 24-hour growth in thioglycollate of a toxigenic strain (obtained from Dr. Milan Logan, Prof. of Biochemistry at the University of Cincinnati Medical School, Cincinnati, Ohio) which will not grow on the usual media except blood agar. Counts cannot be made because the deep colonies in a blood agar plate cannot be seen. The cloudiness of the thioglycollate culture was adjusted to approximate that of an *E. coli* standard so that some billions of *Clostridia* were injected.

**Gas production in thioglycollate was very slight and direct smear showed a few bacteria per oil immersion field.

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TABLE VI

Blood Clearance and Eventual Disposition of Bacteria Injected Into Dogs in Hemorrhagic Shock 1 Hour After Shock Was Induced, Transfused After 2 Hours in Shock

Exper. No.	No of Bacteria Injected (billion)	No. of Bacteria/ml. of Blood: At Beginning (millions)	At 6 hr	Cultural Data: Blood	Spleen*	Liver*	Surv Time, hr†
	Staphylococcus aureus						
1	15	10	200	24 hr +	+	+	24
2	15	10	20	24 hr +	+	+	24
3	45	30	6	18 hr +	+	+	18
4	45	26	4	24 hr +			
				48 hr +			
				72 hr +	+	+	72
5	35	24	40	24 hr +			
				48 hr +			
				72 hr +			
				96 hr +	+	+	96
6	35	17	4	24 hr +			
				72 hr +	+	+	72
7	75	50	20	+	+	+	24
	E. Coli						
8	19	8	20	24 hr +			
				48 hr +	+	+	48**
9	19	8	Sterile	24 hr —			
				48 hr +	+	+	48
10	12	8	5000	+	+	+	24**
11	12	8	50	+	+	+	18
12	17	8	2	24 hr +			
				48 hr +	+	+	48
13	17	8	8	24 hr +			
				48 hr +	+	+	48
	Clostridia						
14	?	?	+				6
15	?	?	+	24 hr +			
				48 hr +	+	+	48**
16	?	?	+	24 hr +			
				48 hr +	+	+	48**
17	?	?	+	24 hr +	+	+	24
18	?	?	+	4 hr +			
				48 hr +	+	+	48

*Cultures of spleen and liver taken at death or as soon as possible thereafter
†The figures in this column are only approximate. Death occurred at or some hours preceding the time given
**Killed when obviously very ill

Reprinted, by permission, from Schweinburg, F E., Frank, H A. and Fine J Bacterial factor in experimental hemorrhagic shock. Evidence for development of a bacterial factor which accounts for irreversibility to transfusion and for the loss of the normal capacity to destroy bacteria. *Am J Physiol* 179 532 (1954)

TABLE VII

Blood Clearance and Eventual Disposition of Bacteria Injected Intravenously Into Dogs 1 Hour After Inducing Hemorrhagic Shock Made Irreversible to Transfusion

Exper No	No of Bacteria Injected (billion)	No. of Bact./ml. of Blood		Survival Time (hr)
		At Beginning (million)	At 6 hr	
	E. Coli			
1	11	9	100	24
2	10	5	3 000 000	8.5
3	18	17	30 000	8
4	16	6	100	10
5	10	7	Sterile	6
6	10	6	Sterile	7
7	30	28	800 000 000	6
8	9	5	56,000 000	8
9	17	6	64 000 000	9
10	18	1	32,000 000	6
	Staphylococcus aureus			
11	9	4	Sterile	6
12	7	3	180	6
13	13	7	20	9
14	13	8	30,000	6
15	42	27	600	10
16	42	29	8	24
17	44	25	5	6
18	44	23	Sterile	6
	Clostridia			
19	?	?	+	4

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cent survived but every 2 hour shock dog which received mash of the liver from a shocked dog died (Table VIII)

TABLE VIII

Intraperitoneal Injection of Dog Tissue Mash Into Normal Dogs and Into Dogs in Hemorrhagic Shock Transfused After 2 Hours

Ex per No	Source of Tissue Mash	Test Animal	50-ml Dose		100-ml Dose		150-ml Dose	
			No	Mortality (per cent)	No	Mortality (per cent)	No	Mortality (per cent)
1	Liver—normal	Normal	6	0	6	33	6	33
2	Liver—shock	Normal	7	0	7	29	8	25
3	Liver—normal	Shock					10	10
4	Liver—shock	Shock					10	100
5	Liver—shock	Shock (℞ penicillin)					10	30
6	Liver—shock (℞ penicillin)	Shock					10	50
7	Muscle—normal	Shock					5	20
8	Muscle—shock	Shock					5	100
9	Liver—shock	Shock (℞ antitoxin)					5	100

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Therefore there is something in shock liver mash which is not present in normal liver mash *Clostridia* are present in both There must be something in addition in shock liver mash that kills That this is a bacterial factor is demonstrated by the next series of experiments In these the 2-hour shock dog received penicillin before it was put into

shock. It received the mash of liver from a shocked dog 1 hour before transfusion. Seventy per cent of these dogs survived. Therefore there is a bacterial factor in the shock liver mash and this factor develops in consequence of the fact that the animal from which it was taken has been in shock.

This bacterial factor, a toxin, is present not only in the liver but also in muscle, because normal muscle was well tolerated by the 2-hour shock dog, but a mash of muscle from a shocked dog was lethal. Experiments now in progress show that penicillin also protects the recipient of shock muscle mash. A test to see if the *Clostridia* are involved was made by additional experiments in which polyvalent Clostridial antitoxin was given. It did no good. The action of neomycin is still the most reliable evidence against a key role for the *Clostridia*.

Dobson: Dr. Fine, it disturbs me that the shocked animals seem to survive better than the normal animals when they have gotten normal liver mash. You get 90 per cent survival in shocked animals with normal liver mash and 70 per cent in normal animals with normal liver mash.

Fine: I do not wish to try to explain that difference. I believe that in a biological assay we can assume that such differences are not substantial. In any case, the antibacterial potential of different animals within the same species varies. That is something about which Dr. Pillemer will have a good deal to say later.

Fremont-Smith: Approximately how many animals were there in these experiments?

Fine: There were ten single experiments for each observation.

Green: When were these injections given with respect to the bleeding curve?

Fine: They were given during the hypotensive period. The dog was transfused after 2 hours of shock. It is important to remember that this is a reversible degree of shock from which such dogs, if left alone, will recover. But the liver mash was taken from an animal which was dying of prolonged shock and which had failed to respond to transfusion.

Green: When was he given this injection?

Fine: After he had been in shock for about 1 hour.

Green: You mean 1 hour after he was bled down to the hypotensive level?

Fine: Yes.

Haist: When this substance is injected does it have any effect on blood pressure?

Fine The blood pressure is kept at 30 mm. Hg until the end of 2 hours and then the animal is transfused

Haist When you put in this liver mash, does it affect the amount of blood that must be removed in order to keep the blood pressure at 30 mm. Hg?

Fine No, we noticed no significant change in the amount of blood in the reservoir

Zweifach Your data indicate that a third of your animals died with normal liver mash

Fine About 30 per cent of normal animals die whether given normal or shock liver mash We find some bacteria in the peritoneal cavity of these animals Of course there is a certain amount of toxin, perhaps bacterial toxin, even in normal liver, but the bacteria we isolate may come from the recipient dog s gut by transmural migration in response to the irritant properties of the liver mash

Shorr Have you ultrafiltered this liver mash?

Fine No

Shorr Do you know whether heating it would destroy its activity? In other words, have you differentiated between live bacteria products of tissue breakdown, and toxins?

Fine No we have not, but there is evidence that there is no significant amount of preformed bacterial toxin, because if it were preformed the penicillin would not protect *

Shorr I would not feel just philosophically that one can be so arbitrary because penicillin may protect an animal being jeopardized by an injection of a toxic substance

Fine I would have to agree to that

Green How do the 30 per cent die infectious death or shock death?

Fine They die in shock

Shorr Would you describe what happens? Have you measured the blood pressure?

Fine We have not measured the blood pressure They simply go into collapse and show the typical cold extremities and lack of urine That does not take place right away It takes a number of hours These animals are dead in less than 24 hours

Horvath The normal animals given injections of normal liver go into shock?

Fine Yes they seem to peter out within 24 hours also

Green Do they have hemorrhage into the gut?

---

*Recent data (3) support the view that antibiotics can protect against bacterial toxins apparently by suppressing bacteria otherwise activated as a result of injury to the bactericidal mechanisms by the toxins

Fine I cannot tell you about that detail in these experiments

Shorr In other words we really do not know exactly what these animals are dying from

Fine They die within 24 hours and seem to be in shock before they die.

Bradley Have you treated these figures statistically? This is a very small series

Fine There are ten experiments for each observation and the results are sufficiently uniform

I wish to make one further point Only 200 gm of shock liver kill a 2 hour shock recipient The animal from which the liver was taken was exposed to a great deal more of the bacterial factor than the recipient the donor was exposed to toxic substances from his entire liver and from his whole body musculature which is also poisonous He was presumably being overwhelmed by toxin Since the donor dog would have survived had an antibiotic been given in advance it follows that the irreversibility to transfusion in the donor was caused by a bacterial factor With these observations in hand we then went on to study the bactericidal mechanisms

Haist The fact that the liver mash gives an effect does not necessarily mean that the materials of the mash would be liberated in the intact animal does it? If tissue is broken up mechanically materials could be liberated from cells that would not necessarily be liberated from the intact tissue in the body

Shorr You mean in the animal from which the liver has been taken?

Fine I will make only the obvious inference from these data. The effect of a mash of normal liver prepared with sterile precautions is compared with the effect of a mash of liver from a dog dying of shock prepared in precisely the same fashion

Fremont Smith Dr Fine, it was a different question he was raising The point Dr Haist is making is it is not necessary to assume that the whole liver intact and not mashed would release these substances and therefore the animal might not be exposed to toxic substance from the whole liver in the same sense as he is exposed to material from the 200 gm of mashed liver Isn t that your point?

Haist That is right

Fine Perhaps not I do not know I think I can say that if a bacterial factor is in the donor s liver it is very likely to get into the circulation

Shorr When you remove the liver from the animal do you freeze it at once?

Fine Yes immediately

Shorr You have in the shocked animal a liver that has been profoundly hypoxic for 2 hours while in the normal animal you have taken out a healthy aerobic liver so that from the metabolic standpoint as well, they are not alike

Fine No of course. We are talking about shock as it affects the liver We take the liver out of an animal dying of shock which is irreversible to transfusion and we compare its action to that of a liver from a normal animal

Nickerson There is no question but that animals which have been hemorrhaged or beaten in a Noble-Collip drum are more sensitive to many things This factor probably is involved in limiting the range of dosage of various agents which will protect against shock. If the dosage is just a bit too high the toxicity of the drug itself may be superimposed on the effects of the shocking procedure even when the dose is well below that toxic to normal animals I hope you will point out as you go along any evidence that this increased sensitivity to bacterial products is really different from increased sensitivity to many things.

Fine I will refer to that later on.

Burch When did you administer the penicillin to the animals receiving the shock liver mash?

Fine This penicillin is administered to the recipient animal prior to exposing it to 2 hours of shock. I did not give data on the effect of giving penicillin to the donor animal We did that too and there was protection. We did not give the donor as much penicillin as we would to prevent irreversibility to transfusion because our objective was to use liver from an irreversible preparation. So we gave the donor partial protection

Burch It was effective either way?

Fine Either way

Burch Did you add penicillin to the mash directly?

Fine We did not give penicillin in the mash

Burch In other words you are not of the opinion that there was a purely chemical effect?

Fine I will deal with that point later (4) In our investigation of the various components of the bactericidal mechanisms we first studied phagocytosis by polymorphonuclear leukocytes Leukocytes from a normal animal were exposed to serum taken at successive periods from an animal in shock, i e., after 2 hours in shock and again after 4 hours 6 hours, and 8 hours The serum was mixed with the leukocytes and with bacteria, and the mixture was incubated for 2 hours at 37 C. (Table IX)

TABLE IX

Percentage of Phagocytosis by Leukocytes from Normal Dogs in Serum of Dogs in Irreversible Hemorrhagic Shock*

Time Blood Taken	Experiments				
	1	2	3	4	5
Before shock	72	90	68	42	40
In shock					
2 hr	80	35	70	38	50
4 hr	76	35	25	40	38
6 hr	28	30	3	38	26
8 hr	22	—	—	26	22

*Percentage of active leukocytes was reasonably uniform, so that the drop in phagocytic count must be attributed to the serum. The protein content of the serum is not significantly different from that in the serum prior to shock. Death occurred shortly afterward in first three, and some hours later in last two experiments.

Reprinted, by permission, from Schweinburg, F B. Yashar Y., Aprahamian, H. A. Davidoff D., and Fine, J Resistance to bacteria in hemorrhagic shock. I Decline of phagocytosis-promoting capacity of serum in shock. *Proc Soc Exper Biol & Med* 88, 587 (1955)

The percentage of cells which were ingesting bacteria remained fairly uniform all the way *But the percentage of bacteria to which they were exposed began to fall in preparations of cells in serum taken after the second hour in shock* In serum taken before shock in one experiment the cells ingested 72 per cent of the bacteria to which they were exposed, whereas in serum taken 8 hours after shock had been induced the cells ingested only 22 per cent

In other experiments the fall was from 90 per cent before shock to 30 per cent after 6 hours in shock, and from 68 per cent before shock to 30 per cent after 6 hours in shock in another the change was from 42 to 26 per cent which is perhaps not a significant change

Zweifach Where in the time course was the transfusion admin

istered? The table shows 2 hours 4 hours 6 hours 8 hours Were they transfused somewhere along the line there?

Fine No The preparation consists of leukocytes which were taken from a normal dog and were immersed *in vitro* in serum taken from shocked dogs The latter are allowed to go into irreversible shock and to die without transfusion. The serum is taken from those dogs after 2 hours in shock, after 4 hours in shock, and so on.

Knisely Bacteria are put in the serum?

Fine Bacteria, leukocytes, and serum are mixed.

Knisely Where do you get the bacteria from, a culture?

Fine A given number from a 24-hour culture

Knisely Every one has the same concentration of bacteria?

Fine Yes

Knisely Were the tubes rolled?

Fine The tube is shaken frequently during the 2 hours of incubation We next investigated (Table X) sensitivity to the bacterial toxin For

TABLE X

Comparative Action of *E coli* Endotoxin Injected I.V.*

Dose in mg/kg	Normal Rabbits	2 Hour Shock Rabbits Time of Injection after Transfusion in Hours				
		4	15	24	48	72
Min lethal (100 per cent)	0.13	0.000001	>0.5			
Max surviving (100 per cent)	0.05	0.00000012	<0.012	<0.023	0.05	0.05

*Each figure represents the dose in mg given to each of 10 rabbits.

Reprinted, by permission, from Schweinburg, F B., and Fine, J Resistance to bacteria in hemorrhagic shock. II Effect of transient vascular collapse on sensitivity to endotoxin. *Proc Soc Exper Biol & Med* 88, 589 (1955)

this purpose, dogs are not suitable In rabbits the intravenous M.L.D / 100 of an *E. coli* endotoxin prepared by Dr Abraham L. Braude (of the Southwestern Medical School of the University of Texas at Dallas) was 0 13 mg /kg and the M.S.D /100 was 0 05 These values were confirmed in many rabbits perhaps several hundred by now all of the same breed and from the same farm.

The M L.D /100 of this toxin was tested in the rabbit exposed to 2 hours of hemorrhagic shock. The shock experiment was done as in the dog except that the lowest blood pressure the rabbit can tolerate is about 50 mm Hg Such rabbits like dogs transfused after 2 hours in shock, recover but 4 hours later when the rabbit is eating and appears fully recovered, it is killed by 1/100 000 of the dose / kg required to kill the normal rabbit If instead of the minimal lethal dose, we determine the maximal survival dose, we find it is 0 05 mg in the normal rabbit but it is 000 00012 in the shock rabbit i e. the 2-hour shock rabbit 4 hours after it has been transfused is from 100 000 to 1 000 000 times more sensitive to a bacterial toxin than it was in the healthy state. The recovery of normal resistance to toxin requires about 48 hours

Zweifach I should like to come back to the point concerning the transfusion of white blood cells from a reservoir in which the blood has remained outside the body for periods up to 4 hours Is it not possible that these white cells have become altered during this period and no longer can effectively serve in the defense against agencies such as bacteria or endotoxins?

Pillemer What evidence do you have for that statement?

Fine The toxin is taken up by the polymorphonuclear leukocytes, by the macrophages and by the reticuloendothelial system in the lungs, liver spleen, and most of the tissues But we have transfused with fresh blood too and have found no difference in the results

Zweifach Some investigators believe that circulating endotoxins are removed in part by the polymorphonuclear leukocytes (5)

Engel If your whole system is siliconed, wouldn t you get around the problem of the white cell survival in the transfusion setup?

Fine The best answer I can give you is that when we transfuse animals with fresh blood we get the same effect

Nickerson Is it not true that these animals were reinfused at about the time the reduction in phagocytic activity occurred? We will not argue now about whether these events are causally related

Shorr In other words the reduction manifests itself for the first time, after infusion?

Fine They were transfused after 2 hours of shock The blood was

in the heparinized buret at room temperature for 2 hours and the rabbits were exposed to the toxin 4 hours later, when their hemodynamic status was back to normal

Nickerson I am talking about the phagocytosis experiment in which you took samples of blood for testing at intervals during the experiment The break to a lower phagocytic index occurred between 2 and 4 hours which was shortly after the reinfusion.

Fine The phagocytosis experiment was done on two groups of dogs In one group the transfusion was given after 2 hours In the second group, no transfusion was given and the shock continued Serum was taken at intervals from the circulation of the dogs in both groups and the cells used for testing this serum were cells from a normal animal

Shorr Could I just go back for one moment to the experiments in which you removed the liver from animals pretreated with penicillin?

Fine The liver from such dogs was not nearly so poisonous to recipient 2 hour shock dogs

Shorr Were these from the animals in your colony which recently showed an entirely different pattern, animals that penicillin did not protect?

Fine These are not dogs tested for the effect of prophylactic antibiotic therapy upon the development of irreversibility to transfusion and upon the survival rate They are dogs given penicillin in advance to subdue without wholly suppressing bacterial activity The aim was to protect them partially but with the deliberate intention of letting them become irreversible to transfusion.

Shorr These were done 4 years ago?

Fine They were done 2 years ago

Shorr Have you done any more recently in animals not protected even though injected with penicillin?

Fine Yes we repeated a few experiments on the effect of prophylactic antibiotic therapy I will discuss this later

I want to refer now to studies of the response of normal rabbits and rabbits transfused after 2 hours in shock to the administration of beef infusion broth into the peritoneal cavity We wanted to compare the cell mobilization capacity of the normal and the 2 hour shock animal (Table XI)

For control purposes we first studied the undisturbed peritoneal cavity Rabbits were killed six hours after transfusion for shock of 2 hours duration Normal rabbits were also killed Immediately after death the peritoneal cavity of each rabbit was washed with 500 ml of Ringer s gelatin solution at 40 C. The number of cells recovered averaged about 3 4 million in both normal and shocked rabbits Seventy per

TABLE XI

Response of Normal Rabbits and of Rabbits Transfused After 2 Hours of Hemorrhagic Shock to Intraperitoneal Beef Infusion Broth 6 Hours After Injection

Intraperitoneal Stimulus	Cellular Response in Normal Rabbits—Per Cent of			Total in Millions	Cellular Response in Shocked Rabbits—Per Cent of			Total in Millions	Remarks
	Macrophages	Polys	Lymphocytes		Macrophages	Polys	Lymphocytes		
None	65*	20	15	3 3	75*	20	5	3 9	Bacteria in three of six shocked rabbits None in unshocked rabbits.
Beef Infusion Broth	7†	93		103	75†	25		4 2	Twice as many bacteria as cells in eight of eighteen shocked rabbits None in unshocked rabbits

*Chiefly immature macrophages
†50 per cent mature macrophages

Reprinted, by permission, from Rutenburg S., and Fine, J Host resistance to bacteria in hemorrhagic shock V Mobilization of phagocytes. *Proc Soc Exper Biol & Med 91 217 (1956)*

he cells were macrophages, 20 per cent were polymorphonuclear tes and the rest lymphocytes

bacteria in and outside the cells were seen in the suspensions from the shocked rabbits None was seen in the suspension from the normal rabbits

onsider the macrophages to be traumatically desquamated peri ming cells They showed ameboid activity looked like macro- and acted as phagocytes

t is the cellular response 6 hours after injecting beef infusion nto the peritoneal cavity of the normal rabbit? By the same g technique we recovered an average of 100 million cells, of 93 per cent were polymorphonuclear leukocytes The rest were hages and of these there were double the number obtained from hallenged peritoneal cavity

ntrast, the cellular response in the 2 hour shock rabbit is virtually ie number of cells is no greater than in the unchallenged peri cavity This animal cannot mobilize macrophages from local ors and it cannot deliver polymorphonuclear leukocytes from the ion. It is important to remember that this is at a time when the ynamic status is back to normal I offer the suggestion that this because something is wrong with the cell permeability promoting discovered by A. A. Miles (6)

t Do you know that the splanchnic circulation has returned to at this time?

I will risk making the assumption that 6 hours after a trans for 2 hour shock the hemodynamic state is quite close to the l in all parts of the circulation

t, we wished to measure the capacity of the phagocytes to lyse a. The cells from normal rabbits (mostly polymorphonuclear ytes) were suspended in normal serum. About 10 per cent of cells ingested bacteria, and of those ingested 90 per cent were by the techniques of Smith and Wood (7) to have been killed 2 hours (Table XII)

same cells were then put into serum taken from the shocked l 6 hours after transfusion This serum injured the normal cells trast to their normal appearance when suspended in serum from nal rabbit, these were shrunken, the nuclear membrane was less efined, and the cytoplasm appeared reduced in amount Ninety nt of them phagocytosed bacteria, but only 20 per cent of the ia ingested were lysed.

me Dr Fine, has your figure that 90 per cent of the normal cells

TABLE XII

Destruction of Bacteria by Phagocytes in Serum From Normal and Shocked Rabbits

No and Type of Experiment	Type of Cells (per cent)	Cells with Ingested Bacteria (per cent)	Bacteria Ingested* (per cent)	Ingested Bacteria Killed (per cent)
Normal cells** in normal serum	Polys (98)	10	30	60 to 90
Normal cells in shock serum†	Polys (98)	90	20	20
Shock cells** in normal serum	Macrophages (80)	30	50	20 to 40
Shock cells in shock serum	Macrophages (80)	30	20	20

*Number of bacteria in Exps. 2 and 4 were far greater after one hour of incubation than at the beginning. The number of bacteria in Exps. 1 and 3 after one hour of incubation were not increased.

†Serum taken from rabbits 6 hours after transfusion for shock of 2 hours duration.

** Phagocytes were removed from peritoneal cavity 6 hours after injection of 50 cc of beef infusion broth In the shocked animals the injection was made just after transfusion.

in shock serum have ingested bacteria as against 10 per cent of the normal cells in the normal serum any significance?

Fine No We found that the bacteria multiplied rapidly in the *normal cell shock serum preparation so that more than twice* as many bacteria were present per high-power field than in the normal cell normal serum preparation The surface contact of cells and bacteria is, *therefore greater The significant fact was the* loss of the ability to kill ingested bacteria

Nickerson Is it possible that the difference in percentage destruction of *ingested bacteria is because of the fact* that many cells which would neither ingest nor lyse bacteria in normal serum do ingest them after exposure to shock serum? Your data indicate that in the first group *only 10 per cent of the best* polymorphonuclear leukocytes ingest

bacteria and these lyse them very well However in the shock group it appears that all of the polymorphonuclear leukocytes ingest bacteria Perhaps many of these extra cells would not have been capable of lysing bacteria in normal serum

Horvath Dr Fine, I am curious about the term shock serum. That is not actually shock serum

Fine It was serum taken 6 hours after a therapeutically effective transfusion

Horvath You have replaced a fair quantity of that with the so-called normal blood that you withdrew earlier or replaced with freshly drawn blood.

Fine The transfused blood usually was the rabbit s own blood. Occasionally a healthy rabbit s blood was used for transfusion. In this case it happened to be the rabbit s own blood

Horvath Shouldn t that serum, then be represented as fairly normal serum instead of so-called shock serum?

Fine It is serum taken from a rabbit 6 hours after it was transfused for the treatment of 2 hours of hemorrhagic shock. That is all I can tell you.

Burch Did you try the serum that you employed for transfusion?

Fine No We have done the following Normal cells were exposed to normal serum from the same and from other normal rabbits There was no difference in the result Normal cells were exposed for 1 hour to serum taken from the shocked rabbit 6 hours after transfusion The cells were washed free of this serum and resuspended in normal serum and then exposed to bacteria They then behaved as if immersed in the serum from a shocked rabbit This demonstrates that the multiplication of bacteria in shock serum does not account for the injury to the cells It is clear that there is a toxin in shock serum and that this is a leukotoxin that prevents lysis *

We then tested the macrophages from the shocked rabbits in the manner described for polymorphonuclear leukocytes from the normal rabbits † Suspended in normal serum they lysed from 20 to 40 per cent of the ingested bacteria Suspended in serum from a shocked animal they lysed only 20 per cent. These are still preliminary data They suggest that the macrophages are injured as a result of the shock process because W B Wood, Jr has data** which indicate that macrophages will lyse as rapidly as polymorphonuclear leukocytes. But it is

---

* More recent data show that the serum in the elevated reservoir as well as the animal's circulating serum, is toxic.

† To complete the comparisons we are currently testing polymorphonuclear leukoyctes from shocked rabbits and macrophages from normal rabbits.

** Personal communication.

possible that macrophages are not as capable of lysis as the polymorphonuclear leukocytes are I am not sure of the facts, and I do not want to make too much of these data. We are getting more information

Zweifach Is it possible to state unequivocally that restoration of blood volume returns all relevant factors to normal in these dogs? As an example, these animals have received considerable doses of heparin.

Fine We use the least possible amount of heparin.

Zweifach There may be other changes in these animals which have as yet not been measured You have indicated one possibility in this regard a fall in prothrombin

Fine This is shock certainly

Zweifach Have you done analysis on the shock serum in terms of other constituents?

Fine Only the ones I have mentioned and properdin. Before continuing I should like Dr Pillemer to tell us about properdin, I have asked him to give us a general discussion as a background for the data we shall present jointly then we might follow with a closing discussion

Pillemer Properdin will be designated as P zymosan as Z. Complement will be termed C′ and its four components C′1 C′2, C′3 and C′4

The normal mammalian serum kills certain bacteria neutralizes viruses and hemolyzes certain abnormal red cells in the absence of demonstrable antibody During the past 20 years we have been trying to learn what serum factors are involved in these phenomena We now believe that the properdin system (8) is in part, responsible for the bactericidal virus neutralizing and hemolytic activities of normal serum. The properdin system consists of properdin complement, and magnesium. This system *in vitro* kills bacteria neutralizes viruses and also hemolyzes certain red cells

Table XIII shows some of the properties of human properdin (8) This is a very large protein molecule, with a sedimentation constant of about 27 S which indicates it is about twenty times the size of albumin It is present in human serum in very small amounts It also is not amylase esterase or lipase

Table XIV shows that properdin is not an antibody (8) Antibodies combine with antigens in the absence of either magnesium or complement and do not require elevated temperatures or a very narrow *p*H range whereas for properdin to combine zymosan or bacterial cell walls magnesium complement, elevated temperature and a narrow pH range are necessary

Table XV shows that if properdin is removed from serum by zymosan at 17 C., none of the factors that are concerned with specific

TABLE XIII

Properties of Purified Human Properdin

Euglobulin with minimum solubility between pH 4 8 and 6 5 Sedimentation constant about 27 S
Represents not more than 2 to 4 μg of protein nitrogen per milliliter of serum, or about 0 03 per cent of the serum proteins
Stable to heating at 66°C for 30 min
Destroyed at 100°C in 5 min
Not a component of the blood-clotting, plasmin, or hemolytic complement systems
Participates in bactericidal, virus-neutralizing, and hemolytic reactions in the presence of complement and Mg^{++}

Reprinted, by permission, from Pillemer L. Blum, L. Lepow I H. Ross, O A., Todd, E. W., and Wardlaw A. C. The properdin system and immunity I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science* 120 279 (1954)

immunity, clotting, or complement activity is removed. However, the heat labile bactericidal activity the heat labile virus neutralizing activity and also the hemolytic activity for unsensitized cells are removed Zymosan is an insoluble glucose polymer from yeast In this discussion, the term is used in a generic sense to indicate any polysaccharide which will complex with properdin. Such products can be obtained from practically all bacteria, and even from mammalian tissue.

Levine There is a formation then of a protein carbohydrate complex?

Pillemer Yes The complex can be centrifuged out of serum

Table XVI shows the strains of bacteria (9) which are killed by the properdin system It is evident that within a species of bacteria some strains are susceptible that is they are killed Some strains are resistant and others are killed even in the absence of properdin As with antibiotics, there are also resistant strains to the properdin system. It is of interest that the properdin system kills for the most part only gram negative bacteria.

TABLE XIV

A Comparison of the Factors and Conditions Required for the Combination of Human Properdin with Zymosan and Antibody with Antigen

Properdin with Zymosan		Antibody with Antigen	
Requires	Mg^{++} ions	Does not require	Mg^{++} ions
Requires	Complement	Does not require	Complement
Requires	Temp >10 C	Does not require	Temp >10 C
Requires	pH 6 5 to 8.2	Does not require	pH 6.5 to 8.2
Requires	$\mu^* = <0\ 4$	Does not require	$\mu = <0\ 4$

*μ = ionic strength

Reprinted by permission, from Pillemer L. Blum, L., Lepow I H., Ross, O A Todd, E. W and Wardlaw A. C. The properdin system and immunity I Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science 120, 279 (1954)*

Nickerson The common staphylococci and streptococci are resistant to this system?

Pillemer *They are resistant That is a story by itself because these* bacteria produce streptokinase or staphylokinase that destroys complement which is required for properdin activity

Dr Oscar A Ross and Dr Alan R Moritz, *Institute of Pathology Western Reserve University Cleveland are interested in total body* irradiation (8) and it appeared that the properdin system might be *involved in radiation illness* Table XVII shows that following 500 r in rats there is a profound fall in the properdin from 25 to 35 units to under 1 unit at the end of 13 days There is no fall in complement In fact, complement titers increase

We have observed the same fall in properdin *if varying amounts of E. coli* or *S typhosa* or any of the gram negative bacteria are injected into mice If 3 4 x 10 *E. coli* are injected properdin falls to very low values at 24 hours and the animals are dead at 72 hours If 3 4 x 10[10]

TABLE XV

Comparison of the Factors and Activities of Untreated Human Serum and Properdin Free Serum (RP)

Factor or Activity	In Untreated Serum	In Properdin free Serum (RP)
Hemolytic activity for sensitized sheep cells	Present	Present
Individual complement components	Present	Present
Susceptibility to complement fixation	Present	Present
Plasminogen	Present	Present
Plasmin inhibitors	Present	Present
Clotting factors	Present	Present
Hemagglutinin	Present	Present
Susceptibility of C'3* to inactivation by zymosan	Present	Absent
Heat labile bactericidal activity	Present	Absent
Heat labile virus-neutralizing activity	Present	Absent
Hemolytic activity for unsensitized cells	Present	Absent

*C'3 = third component of hemolytic complement.

Reprinted, by permission, from Pillemer L. Blum, L., Lepow I H Ross, O A Todd, E. W., and Wardlaw A. C. The properdin system and immunity I Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science* 120 279 (1954)

organisms are given, the fall in properdin occurs early and the animals are dead within 24 hours

Dr Ross (9) has been able to protect mice against total body irradia

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Requires	$\mu^{*} = <0\ 4$	Does not require	$\mu = <0\ 4$

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Hemagglutinin	Present	Present
Susceptibility of C'3* to inactivation by zymosan	Present	Absent
Heat labile bactericidal activity	Present	Absent
Heat labile virus-neutralizing activity	Present	Absent
Hemolytic activity for unsensitized cells	Present	Absent

*C'3 = third component of hemolytic complement.

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Dr Ross (9) has been able to protect mice against total body irradia

TABLE XVI

Sensitivity of Various Microorganisms to the Bactericidal Action of the Properdin System in Human Serum

Organism	Number of Strains		
	Sensitive to the Properdin System	Highly Sensitive to Both Serum and Properdin-Free Serum (RP)	Insensitive to Serum
Shigella	3	3	
Proteus	2	1	5
Pseudomonas	3		
Paracolobactrum	2		3
Escherichia coli	3		1
Salmonella	3	3	2
Aerobacter aerogenes			2
Vibrio comma		1	
Bacillus subtilis	2		1
Streptococcus faecalis			1
Staphylococcus albus			1
Yeast			1

tion with properdin However the time when it is given is very important If properdin is given too early it is of little or no value and if it is given too late it can be very harmful to the animal

Following 600 r mice were given 50 units of properdin on the

TABLE XVII

The Serum Properdin and Complement Titers in Rats Total Body Irradiated with 500 r

Postradiation Days	Properdin (Units)	C' 100 per cent Hemolytic Units	Percentage of Normal Serum Values			
			C'1	C'2	C'3	C'4
0	25 to 35	30 to 40	100	100	100	100
2	4 to 6	50	100	100	100	200
7	<1	50	100	135	200	200
13	<1	60	100	200	300	200

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second third, fourth fifth, seventh, and tenth day following radiation There was no protection until the injection on the fifth day (50 per cent protection) However if properdin were injected only on the seventh day the animals all died suddenly with what appeared to be an acute endotoxemia.

The properdin titer is very low at the seventh day and these animals have a bacteremia of enteric origin. As soon as properdin is injected, these bacteria lyse and liberate endotoxin.

Engel Can you protect the animal by giving cortisone?

Pillemer Cortisone has no effect on the properdin system

Engel I mean at the point where they get the so-called Herxheimer s reaction Would cortisone protect them against this reaction?

Pillemer I do not know whether that has been done or not

Burch Will antibiotics protect these animals?

Pillemer They will give a small degree of protection if the animals are pretreated for several days.

Fremont Smith Both pretreatment and continued treatment throughout?

Pillemer Yes

Levine Are these days after completing the dose of irradiation?

Pillemer Yes days after irradiation

Zweifach Do any of your animals die by the fourth day?

Pillemer Not at 600 r On the fourth day there are no deaths Control animals died between the sixth and fifteenth days following irradiation

Dobson A characteristic effect of irradiation is that animals exposed to an LD_{50} do not die until after the first week and then those that are going to die have usually died by about the fourteenth day

Zweifach A Wistar strain of rats used in our laboratory begins to die on the fifth to sixth day with further deaths up to the twentieth day following an LD_{50} dose of roentgen ray

Pillemer Next, we will discuss some things which Dr Fine will also touch on later Since the supply of properdin is limited at this time we tried to find some way to increase the properdin titer in experimental animals It was found that if mice are injected intravenously with zymosan (10) or bacterial cells or endotoxin the properdin titer falls very promptly after the injection The rather sharp fall in properdin titer is then followed by elevated properdin levels

During the phase of low properdin levels animals are very susceptible to infection However during the period of high properdin titers animals are very resistant to infection So there seems to be some suggestion that this fall and rise in properdin is related to the degree of resistance or susceptibility of these animals

Figure 9 shows that the same response can be obtained with other polysaccharides With endotoxin or neutral polysaccharides there is an initial fall in properdin levels during which time the mice are very susceptible to infection This is followed by elevated titers in the case of the levan and typhoid endotoxin This perhaps suggests an explanation for tolerance to endotoxin It also perhaps explains why mucin has been so useful as a virulence-enhancing agent

Engel Does pyromen fit into the picture?

Pillemer Pyrogens are lipopolysaccharides This may also explain some of the beneficial results reported following the injection of pyrogens into humans

Zweifach Are animals with high properdin titers resistant to other forms of experimental stress?

Pillemer Yes Tourniquet and drum shock The experiments on drum shock were conducted by Dr Marion E Webster Walter Reed Army Institute of Research Washington Dr Simon Koletsky Western Reserve University Cleveland is doing the work on tourniquet shock

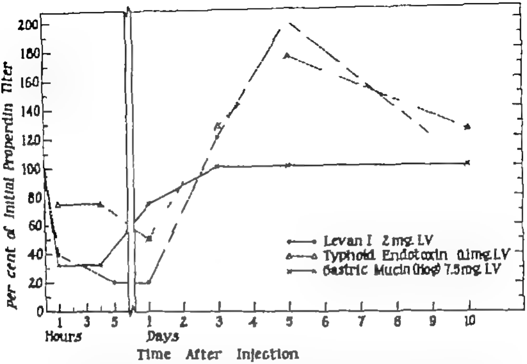

FIGURE 9 The serum properdin levels in mice following intravenous injection of levan, mucin, and endotoxin. Reprinted, by permission, from Pillemer, L., Schoenberg, M D., Blum, L. and Wurz, L. Properdin system and immunity II Interaction of the properdin system with polysaccharides. *Science* 122, 545 (1955)

Zweifach Are animals resistant to drum shock also resistant to the lethal effects of endotoxin?

Pillemer I am presenting work done by Dr Marion E. Webster You will have to accept her experimental data in her absence Rats given 1 mg of endotoxin (Table XVIII) or tumbled 300 turns 4 days prior to challenge with 4 mg endotoxin or 500 turns are substantially protected against these treatments

Zweifach The term drum resistance has been used in the past to indicate a progressive adaptation to a series of sublethal drummings until the rat withstands 1000 drummings a dose which is 100 per cent lethal to normal controls

Pillemer I understand Dr Webster found that 500 turns is an LD [80] to LD [90] with their rats Three hundred turns is nonlethal However 4 days after 300 turns the animals are resistant to 500 turns the time element here is very important.

Zweifach Are they resistant to more than 500?

Pillemer I do not know

Zweifach Our own studies (11) on resistance utilize the procedure

TABLE XVIII

Correlation of Serum Properdin Concentration with Resistance to Trauma in the Rat*

Treatment	Challenge†	Number of Rats		Per cent Mortality		Properdin Titers**	
		Treated	Controls	Treated	Controls	Treated	Controls
Resistant to tumbling shock	8 mg endotoxin	8	8	12.5	75	33	15
Endotoxin 1 mg	1 mg endotoxin	16	16	0	56	41	21
	tumbled 500 turns	32	32	17	87.5	36	14
Tumbled 300 turns	4 mg endotoxin	32	32	28	72	30	20
	tumbled 500 turns	32	32	31	47	29	22
20 mg levan	Tumbled 500 turns	32	32	19	10	4	11

* Unpublished data obtained from Marion E. Webster

† All groups were challenged 4 days after treatment.

** Titers represent averages of sera obtained from 2 to 8 rats bled on day of challenge

first employed by Noble and Collip (12) The resistance which develops following a protracted training procedure over a period of from 12 to 14 days can be sustained indefinitely by exposure to drum trauma at 8- to 10-day intervals Such rats withstand not only 1000 revolutions in the drum, but frequently as much as 1500 without lethal outcome.

Pillemer I do not know whether Dr Webster has done such experiments or not. I am not a physiologist in any sense of the word and have not carried out experiments of this nature. There is however a tie up here in the fact that animals made resistant to tumbling shock by 300 prior turns are also resistant to 8 mg of endotoxin

Zweifach Both 300 and 500 turns in the drum are completely without lethal effect in our own colony of rats Rats reared under different circumstances in various laboratories probably withstand shock to a variable extent, depending on their diet what bacteria they harbor etc

Pillemer You would have to contact Dr Webster about that Her laboratory has had a great deal of experience with different forms of shock.

Burton These rats were subjected to 300 turns you say 300 conditions them

Pillemer Control animals show this In one experiment 87 per cent of their animals died in another 75 per cent and in another 72 per cent The point to observe here is that the mortality in the controls and the treated animals follows the properdin titers

Shorr I think we should let you describe the experiments and then any comments can be entered into the record

Pillemer It is always hard to describe someone else s experiments but I thought they would be of some interest here

As you see, wherever you see an increase in protection, there is always a parallel increase in properdin titers

Shorr Do you have that data from Dr Webster?

Pillemer Yes. This may explain Dr Fine s work where he injected liver brei into dogs

We have obtained through the courtesy of Dr Murray J Shear National Cancer Institute, Bethesda, polysaccharide complexes of mammalian origin which when injected into animals alters their resistance. We feel that in shock there is tissue damage and that polysaccharides are liberated which combine with properdin. This may alter in the total antibacterial potential of the animal Thus properdin could be an index of the total amount of tissue damage.

Horvath I gather the size of the molecule is critical Have you any idea what the size could be?

Pillemer It has to be over 10^7 in molecular weight

Dobson Does dextran interfere with the properdin?

Pillemer Yes We have published work on this (13) However only certain dextrans are active The ratio of 1 3 and 1-4 linkage to 1-6 seems to be important

Horvath Yet they are up in the same electron linkage the 1-6 it isn t molecular size

Pillemer Molecular weight is also a determinant That is the active polymers are all over a million in molecular weight

Horvath The dextran molecules?

Pillemer Yes the dextrans

Burch What is the function of magnesium?

Pillemer I could perhaps answer that if we knew what it does in other enzyme systems

Burch Have you performed tracer studies with magnesium?

Pillemer We have planned such experiments

Fine Figure 10 shows a curve of the properdin titer in the sera of dogs allowed to become irreversible to transfusion for hemorrhagic shock The titer before shock was normal in five of the first six animals studied The sixth had a very low titer We observed a substantial fall in the titer between the second and third hour in all five animals with a normal initial titer The sera were sent to Dr Pillemer in code

It is important to point out that there is considerable variation between individuals within a species as well as from one species to another I will try to point up the significance of this when we discuss the divergences between the data obtained by Hardy and DeBakey (14) and our own data

Horvath Dr Fine is that titer per volume of fluid?

Fine Units per milliliter of serum The complement does not change The properdin remains low long after the transfusion is given

As I stated one of the first six dogs studied had a very low initial properdin titer (Figure 11) This was the only dog that could not tolerate the shock state for more than about 2 hours and had to be transfused at 3 hours after which it died very quickly Whether the low properdin titer was related to this I do not know

Barton Dr Fine could I ask you if after transfusion you would not naturally expect to get a rise in the properdin from the blood you put in? It is the animal s own blood is it?

Pillemer We have wondered about this The properdin titer falls very sharply within an hour You cannot explain this as an inter

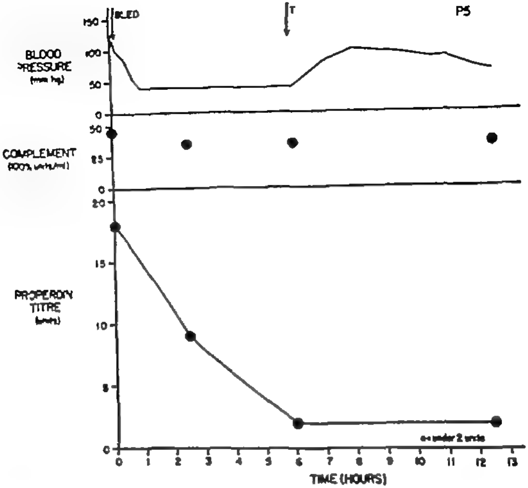

FIGURE 10 Curve of the properdin titer in the sera of dogs allowed to become irreversible to transfusion for hemorrhagic shock. Reprinted, by permission from Frank, E. Fine, J., and Pillemer L. Serum properdin levels in hemorrhagic shock. *Proc Soc Exper Biol & Med* 89 223 (1955)

action of properdin with bacteria or endotoxin Something appears to be released in the host which removes properdin At that time I believe that several thousand units of properdin would have been completely bound

Engel Where is properdin made?

Pillemer I do not know but there is some evidence it is made in the bone marrow

Nickerson The course of the blood pressure in these experiments appears to be quite different from that in your usual shock procedure Did you vary the procedure in these properdin experiments?

Fine No we did not vary the procedure at all

Pillemer These blood samples did not contain heparin.

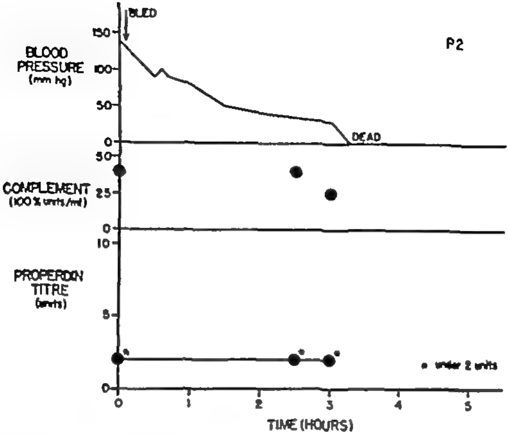

FIGURE 11 Curve of properdin titers in a dog with a low initial properdin titer Reprinted, by permission, from Frank E., Fine J and Pillemer L. Serum properdin levels in hemorrhagic shock. *Proc Soc Exper Biol & Med 89 223 (1955)*

Fine I am wrong I forgot to mention that in order to avoid heparin contamination of the serum we did not use the reservoir technique We withdrew or added blood by needle and syringe to keep the blood pressure between 30 and 40 mm Hg

Nickerson This dog did not reach a pressure of 30 mm Hg for 2 or 3 hours

Fine That is right He died very quickly just the same He was not able to stand the hypotension.

Nickerson However this is very different from the usual procedure of bleeding rapidly to 30 mm. Hg and I am not sure that the results can be compared

Fine This dog had the advantage of a higher pressure From the point of view of blood pressure this dog had matters in his favor rather than against him In the other dogs the blood pressure was maintained between 30 and 40 mm. Hg right along

Engel What is the relation if any between C reactive protein and properdin?

Pillemer None at all

Barton You mentioned this other animal was without heparin Does heparin interact with properdin in some way?

Pillemer It is anti-complementary and interferes with our assays We asked Dr Fine not to use heparin for this reason.

Barton You can add complement?

Pillemer Yes, but it is not too satisfactory

Horvath Is the total amount of heparin important in the reaction of the animal?

Fine I cannot answer that The first group of six dogs in shock assayed for properdin were not transfused (Figure 12) In the subsequent group of 25 dogs the average preshock properdin titer was 12 units per ml Some of these were transfused at the end of 2 hours At about this time the properdin titer had already fallen. These dogs recovered Nevertheless the properdin titer fell still further and remained low for about 4 days and then began to recover There was

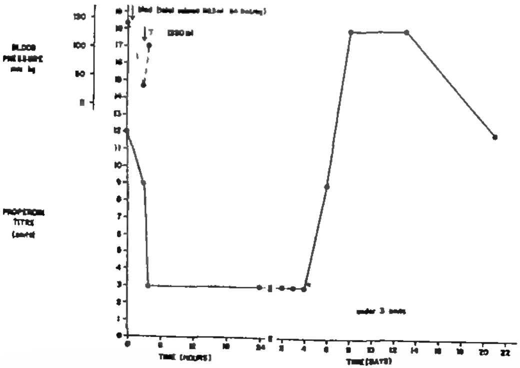

FIGURE 12 Effect of reversible hemorrhagic shock (2 hours at arterial pressure 30 mm. Hg) upon serum properdin. An abrupt and severe decline within 3 hours can be noted, and then recovery with overshoot after 4 days. Unpublished data of Frank, E., Davidoff D., Helfand, Z. Pillemer L. and Fine, J

TABLE XIX

Effect of Hypothermia in Severe and Prolonged Hemorrhagic Shock on Tolerance to *Hypotension and on Survival Rate*

Type of Experiment	Number of Animals	Duration of Hypotension (av hr)	Maximum Bleeding Volume (av ml/kg)	Shed Volume Taken Back Prior to Transfusion (av per cent)	Survival Time of Nonsurvivors (hr)	Survivors
Control—Shock without hypothermia or antibiotic	00+	5	53	40	6	70
Group I—Hypothermia prior to shock no antibiotic	11	7	44	17	30	0
Group II—Hypothermia prior to shock antibiotic* with transfusion and for 3 days thereafter	9	7.3	46	12	0	100
Group III—Hypothermia after induction of shock, antibiotic with transfusion and for 3 days thereafter	10	7.0	60	26	31	30

* Penicillin and streptomycin

Reprinted in part, by permission, from Friedman, E. W., Davidoff D., and Fine J Hypothermia in hemorrhagic shock *Am J Physiol 185 321 (1956)*

an overshoot between the fourth and the eighth days, and eventually the titer fell to normal

Burch Where does the properdin go? It disappears very rapidly

Pillemer We feel that it is either bound to some substance that is exposed in the tissues following shock, or that tissue products appear in the serum which combine with properdin. Dr Fine and I intend to label properdin and really find out where it goes in these animals

Burch Is it found in the urine?

Pillemer No properdin is not found in urine It is not in any other body fluid except serum

Kinsely What did you say the molecular weight is?

Pillemer About 1½ million

Burch Of course, red cells escape into many body fluids

Pillemer That is right, but we still haven t detected properdin in any other body fluid except blood.

Fine Hemorrhagic shock experiments were then done to see if hypothermia would preserve the enzyme systems We used precooling and also cooling started after shock was induced It should be remembered that 90 per cent of normothermic animals exposed to hemorrhagic shock for from 4 to 5 hours and then transfused die. Furthermore, they are not benefited by an antibiotic given at the time of transfusion. I mention this again to emphasize that the timing of antibiotics like the timing of hypothermia or sympathetic blockading drugs, is of tremendous importance in the therapeutic result of transfusion (Table XIX)

Kinsely Dr Fine, from that very fact alone would you suggest giving an antibiotic to soldiers going into battle?

Fine I would answer this with a qualified affirmative, but prefer to leave it for a more extended comment later in the discussion.

As the data show precooling alone enabled the dog to tolerate the hypotension for much longer than the normal period of time. Instead of death after an average of 6 hours these dogs appeared to recover from shock only to die after an average survival time of 30 hours

The hypothermia produced a substantial degree of protection Why did they die? We had reason to believe that this was a result of bacterial action. We tested this idea by repeating the experiment and giving an antibiotic at the time of transfusion so as to protect the dog in the posttransfusion phase against bacteria we got 100 per cent recovery Therefore, the hypothermia gave incomplete protection and the further protection required in the recovery phase while the defenses were still down was provided by the administration of antibiotic in advance of the reactivation of bacterial activity

Nickerson How many animals were in these groups?

Fine They are a substantial number and the results are almost uniformly the same in each group

Levine Dr Fine would this suggest to you that the early shock death is not bacterial while the late death is bacterial? Pre cooling allows the animals to survive 30 hours but precooling in addition to an antibiotic given at the time of transfusion which ordinarily is not effective protects them after the first 30 hours

Fine Yes

Levine Would that suggest in general that early shock death is something that can be prevented by precooling while the late shock death has a bacterial element?

Fine I misunderstood you No the bacterial element operates in both cases The time of death depends on the amount of toxin and the rate at which increasing sensitivity to toxin develops

Shorr I think Dr Levine s point is worth further thought

Green Would your animals tolerate 40 mm Hg or whatever the level is for 8 hours before they took up 50 per cent?

Fine They did not take back blood in significant volume even after 8 hours As a result the experiment took all day so instead of waiting to see if decompensation would appear we transfused arbitrarily at 8 hours and obtained a good response only to observe subsequent collapse and death after an average of 30 hours

May I point out now that in the early experiments with prophylactic antibiotic therapy about 15 per cent of the animals died within 36 hours after an apparently curative transfusion If they survived for 48 hours they survived indefinitely

Shorr A description of the way these animals die is needed before we can relate this phenomenon to what is generally agreed upon as death from shock

Fine We have no hemodynamic data from which we can tell whether or not these animals died in shock but they appeared to

Knisely Dr Fine shows clearly the significance of bacteria in the whole phenomenon but the mechanism of death has not yet been under stood by us

Fine I think the death is caused by sepsis Sepsis develops because the bactericidal mechanisms are injured by the shock process Death from sepsis is usually preceded by the development of shock

Haist Would you let us know the nature of the precooling?

Fine Yes These animals were packed in ice under ether anesthesia until the body temperature dropped to 28 C Etherization was then

discontinued and the animal was allowed some 30 minutes to blow it off Thereafter the animal was bled.

Horvath His temperature decreased?

Fine The temperature continued to fall and leveled off at 23°C. It then rose slowly and remained between 25° and 28 C. until transfusion after which it began to rise

Baez. Would you agree that if the precooled animals were transfused with the volume of blood shed, at 4 or 5 hours as the control dogs were, they might have shown a higher survival rate?

Fine These were transfused

Baez. They were transfused at 8 hours?

Fine They were transfused after 8 hours and none survived

Fremont Smith If they were transfused at 4 hours, rather than 8 hours?

Fine Perhaps they would. I am not prepared to say

Pillemer Dr Fine, it seems to me the animals precooled and carried in hypotension for 4 hours all recovered I think you have experiments showing that.

Fine I shall show more data in another table

Nickerson Can the apparent resistance of the precooled animals be related to differences in their primary and secondary bleeding volumes?

Fine Yes there is a difference in the amount of bleeding I shall come to that soon What is the effect of precooling upon the animal s resistance to injected bacteria? As I said before, normal dogs given an intravenous dose of *E. coli* survive unscathed.

But if they are exposed to 2 hours of shock and given the same intravenous dose of bacteria there is a 100 per cent mortality Death occurs from 1 to 4 days later This is true whether the bacteria are given during shock or after transfusion.

When these experiments were repeated in precooled animals 75 per cent survived when the bacteria were injected while the temperature was still low that is to say during the 2 hours of hypotension This could mean that the hypothermia merely inhibited multiplication of bacteria. But when bacteria were injected after the transfusion, i e., when the temperature was back to normal, the animals were also protected Therefore it can be said that hypothermia sustained the integrity of the bactericidal mechanism (Table XX)

Nickerson You indicated that there were significant differences in the bleeding volumes which suggest that 2 hours in shock actually subjected the hypothermic animals to less stress than the animals with

Nickerson How many animals were in these groups?

Fine They are a substantial number, and the results are almost uniformly the same in each group

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TABLE XX

Effect of Hypothermia Upon Resistance to Bacteria Given During or After Shock of 2 Hours Duration*

Group	Experiment	No. of Dogs	Survivors	
			Number	Per Cent
I	*E. Coli* intravenously in normal dogs	10	10	100
II	2 hour shock in normothermic dogs	10	9	90
III	2 hour shock in normothermic dogs *E. coli* intravenously during shock or at any time up to 24 hours after transfusion	30	0	0
IV	Hypothermia (28 C) induced prior to 2 hour shock *E. coli* intravenously during hypotensive period — warmed to normal temperature after transfusion	8	6	75
V	Same as Group IV except *E. coli* given after body temperature was back to normal†	14	11	79

*Hemorrhagic shock—bleeding to blood pressure 30 mm Hg for [illegible] hours followed by transfusion of all shed blood.

†These experiments include—four in which a coagulase-positive hemolytic *Staphylococcus aureus* was used. The recovery rate is about the same for this as for *E. coli* in normothermic and hypothermic dogs.

Reprinted by permission, from Frank, E. D., Davidoff, D., Friedman, E., and Fine, J.: Resistance to bacteria in hemorrhagic shock. IV. Effect of hypothermia on clearance of intravenously injected bacteria. *Proc. Soc. Exper. Biol. & Med., 91: 188 (1956).*

normal body temperature. This difference may be important in interpreting the results

Fine That is true, but we have experiments in which the animals were first bled way down and then cooled These animals also survived for 30 hours The protection in the precooled animal is, therefore, not due to the smaller blood loss

Horvath Is the number of animals you have, Dr Fine, representative of the total number of animals you used in your cooling experiments or are these just those which survived cooling itself? Because certainly the average results of most people in just cooling alone give about 50 per cent survival with temperature down to 23°C.

Fine In what kind of shock?

Horvath Just cooling by itself Yet with shock supplemented by cooling almost all your animals survive that length of time Your percentage is up to 79 Do you think it is the hypotensive state that you have induced which has prolonged or increased the survival rate of even the cooled animals?

Fine We cooled these animals to 28°C. before shock They remained at or below this temperature for only 2 hours I think the mortality rate you have in mind applies to animals cooled for a much longer period of time

Horvath I am rather surprised at that because of the experience of other groups This is the only group of experiments I have seen where survival has been so good

Fine This is the only group in which the cooling was for only 2 hours

Horvath You stopped the cooling at 28 C. and then they coasted down for another 5 degrees?

Fine After they reached 28°C., they were put into shock for 2 hours The temperature during this period fell to 23°C. then rose from 23° to 25°C., and then stayed between 25° and 28°C. At the end of 2 hours they were transfused and the temperature went up

Fremont Smith They were below 28°C. for 5 or 6 hours?

Fine They were below 28 C. for only 2 hours

Burch Dr Pillemer how do you do the assays?

Pillemer We employ the standard zymosan (15) assay and also check samples in the bactericidal assay and against PNH red cells

Burch Do you use human erythrocytes?

Pillemer Yes PNH cells are human cells

Fine The data already presented suggest that hypothermia protects the shocked animal by virtue of its capacity to prevent injury to the enzyme systems involved in the bactericidal mechanisms These data

furnish indirect evidence of the validity of the general thesis proposed There are more direct ways to measure the integrity of the antibacterial mechanisms and one is by the assay of properdin As shown in Table XXI the properdin titer does not fall during shock if the animal is precooled There are only a few such experiments to date * One animal had a low properdin titer to start with, and it remained low

Horvath These experiments differed from others where you only took a single blood volume out, Dr Fine?

Fine No these animals were kept in shock for as long as 6 hours.

Horvath At a mean arterial pressure of 37 mm Hg to 45 mm Hg?

Fine Yes

Nickerson I do not quite understand the procedure by which you induced shock. The mean pressures are considerably higher and there appears to be considerably more pressure fluctuation than in your earlier experiments Do these experiments differ from your earlier ones?

Fine Yes The properdin assay cannot be done on heparinized plasma or serum Without heparin we could not use the reservoir technique The bleeding had to be done by removing blood with a needle and syringe as required to produce a blood pressure of 30 mm Hg By this method one cannot maintain the pressure uniformly at this level, so it fluctuates to some degree

*A subsequent additional series of six experiments gave the same results.

TABLE XXI

Properdin Titers of Dogs Subjected to Hemorrhagic Shock During Hypothermia

Dog No	Body Temp	Bleeding Volume (ml/kg)	Arterial Pressure (mm Hg)		Properdin Titers During Shock		
			Mean	Range	Initial	2 Hr	6 Hr
P 52	under 28°C	37	45	30 to 60	12	12	12
P 53	under 28 C	39	40	30 to 60	12	12	12

Nickerson Your pressures are measured from a base line somewhere in the thorax?

Fine Femoral arterial pressure.

Nickerson The zero point selected is very important in evaluating blood pressure figures in hemorrhagic shock experiments A difference of just a few centimeters in the base line, a few millimeters of mercury in the effective pressure, may determine whether an animal survives, goes into irreversible shock or dies from acute respiratory failure.

Frank These are against heart levels

Shorr Did the last animals survive or die?

Fine The precooled animals?

Shorr Yes

Fine I do not recall We did not do survival studies on these, just properdin assays

I wish, now to say just a little about the cardiovascular dynamics For example Table XXII shows a normothermic animal prior to shock with a cardiac output of 3592 ml./min under ether and hypothermia it fell to 1909 ml /min. Shock was then added and the cardiac output fell to a fantastically low level. The stroke output was 3 ml /minute! The peripheral circulation was almost at a standstill and yet the animal survived after transfusion.

The data I wish now to present show that in the absence of bacterial toxin an animal can tolerate shock caused by loss of a plasma filtrate for a very much longer period of time than it can tolerate shock caused by whole blood loss

Septic peritonitis was induced by the intraperitoneal injection of 15 ml of a fecal suspension Figure 13 shows a typical experiment. The blood pressure began to fall at about 3 hours, reaching 60 mm. Hg at 6 hours The pulse rose and the temperature fluctuated unevenly

There was a severe fall in plasma volume but the red cell volume did not change much Cardiac output fell to a very low level the peripheral resistance increased, and the A V oxygen difference rose substantially The fluid loss was all into the peritoneal cavity Death occurred after about 9 hours of severe hypovolemic shock

When we treated the animal for oligemic shock by fluid therapy alone by 150 ml. increments of plasma given at the beginning of shock and about every half hour thereafter little good resulted The situation was virtually unchanged even though the plasma volume deficit was kept within the nonlethal range Death occurred within 12 hours (Figure 14)

If instead of giving fluid we gave antibiotic in advance of inducing the peritonitis, and nothing else shock developed the hemodynamic

TABLE XXII

Cardiovascular and Respiratory Dynamics in Dogs Precooled Under Ether and Subsequently Subjected to Hemorrhagic Shock

	Rectal Temp (° C)	Arterial Pressure (mm Hg)	Pulse Rate (min)	Cardiac Output ml/min	Arterial O_2 (vol per cent)	A V O_2 diff (vol per cent)	Pulmonary Ventilation (l/min)	O_2 Consumption (min)	Respiration Rate (min)
No hypothermia or shock	38	124	111	3592	22.9	3.7	18.3	97.4	30
Hypothermia (under ether)	28	101	109	1909	23.8	3.4	11.3	50.6	27
Hypothermia and shock (no ether)	21.5	30	70	181	22.9	12.6	1.9	20.5	16
After transfusion and rise in body temperature	33.5	112	95	3355	22.7	5.5	11.8	123.0	18
Number of experiments represented in each value listed		(23)	(23)	(4)	(4)	(4)	(4)	(1)	(23)

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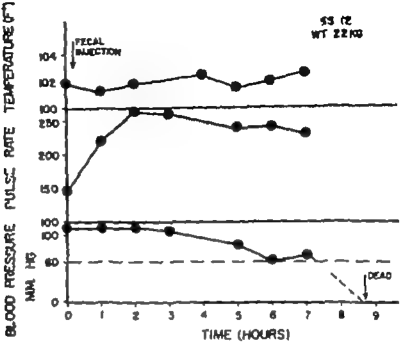


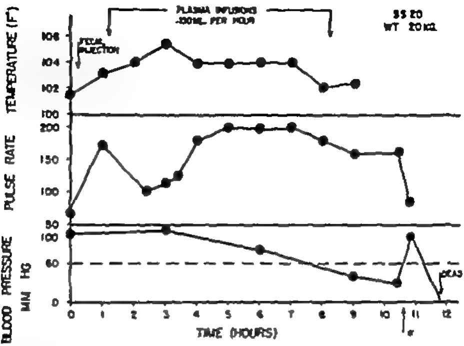
Hematocrit, %	52	64	72
Plasma vol ml	1108	825	689
Red cell vol ml	1217		1034
Cardiac output ml/min	4600	1622	1360
A V oxygen diff vol %	4 1	9 6	13 0
Peripheral resistance (p.r u.)	024	065	048

FIGURE 13 Hemodynamics of untreated hypovolemic septic shock (experimental peritonitis) Representative example. Reprinted, by permission, from Frank, E D Kaufman, D., Korman, H., Schweinburg, F., Frank, H. A., and Fine, J Effect of antibiotics on hemodynamics of hypovolemic septic shock. *Am J Physiol* 182, *166 (1955)*

data were about the same as when no antibiotic was given, the plasma volume fell to 50 per cent of normal cardiac output was way down, and the hematocrit way up The dog remained in deep shock as long as 12 hours but then slowly recovered (Figure 15)

Fremont Smith In shock, by what definition? He hasn t low blood pressure

Fine That is true A well sustained though less than normal blood pressure is characteristic of the early phase of this type of shock as it is of tourniquet shock, and any other type in which the blood volume loss is almost wholly a plasma rather than whole blood loss In the dog treated with antibiotic, the fact that the blood pressure did not fall



Hematocrit, %	51	50	48	44	
Plasma vol., ml	975	964	703	711	
Red cell vol. ml	956		815		
Cardiac output ml/min	4670	2307	1250	1185	1180
A V oxygen dif., vol %	3 4	10 2	11 1	10 2	6 3
Peripheral resistance (p.r.u.)	023	083	064	034	035

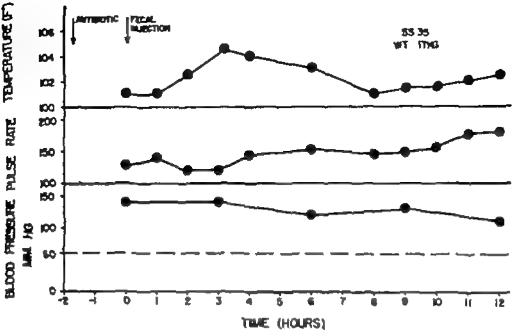
FIGURE 11 Hemodynamics of hypovolemic septic shock (experimental peritonitis) Representative example of effect of plasma infusions. (At the asterisk norepinephrine was administered) Reprinted by permission from Frank E. D., Kaufman, D., Korman, H Schweinburg, F Frank, H A., and Fine J Effect of antibiotics on hemodynamics of hypovolemic septic shock. *Am J Physiol 182, 166 (1955)*

to really low levels suggests that the circulation was not being further damaged by bacterial toxins

Fremont Smith You said he has been in shock 12 hours I do not understand what you mean

Fine The dog loses a tremendous amount of fluid into the peritoneal cavity during the first few hours and there is marked hemoconcentration

Fremont Smith He has not been in shock according to the usual definition of shock



Hematocrit %	52	64	70	73	76
Plasma vol ml	800	613	527	423	466
Cardiac output ml/min	5690	2870	1306	1610	2250
A V oxygen diff vol %	3 5	4 6	9 4	8 2	4.6
Peripheral resistance (p.r u.)	028	040	091	081	040

FIGURE 15 Hemodynamics of hypovolemic septic shock (experimental peritonitis) Representative example of effect of prophylactic antibiotic therapy Reprinted, by permission, from Frank, E. D., Kaufman, D., Korman, H. Schweinburg, F Frank, H. A., and Fine, J Effect of antibiotics on hemodynamics of hypovolemic septic shock. *Am J Physiol* 182, *166 (1955)*

Fine I am defining the characteristics of one kind of shock, the kind we are dealing with here.

Fremont Smith A new definition of shock?

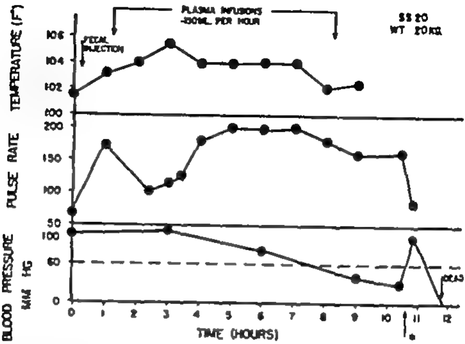
Fine No What I am saying is that in terms of flow through the peripheral circulation, this animal was severely deficient.

Fremont Smith The same kind of peripheral flow as if he had been in shock?

Fine I stated here several years ago that by the term shock I meant an acute and persisting deficiency of flow through the peripheral circulation

Fremont Smith Irrespective of blood pressure?

Fine Irrespective of blood pressure. In these terms the data bear out that definition



Hematocrit, %	51	50	48	44	
Plasma vol ml	973	964	703	711	
Red cell vol., ml	956		813		
Cardiac output ml/min	4620	1392	1150	1185	1180
A V oxygen diff., vol. %	3 4	10 2	11 1	10 2	6 3
Peripheral resistance (p.r u.)	023	053	064	011	085

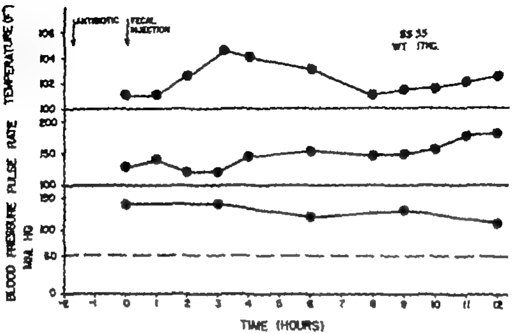
FIGURE 14 Hemodynamics of hypovolemic septic shock (experimental peritonitis) Representative example of effect of plasma infusions. (At the asterisk norepinephrine was administered.) Reprinted, by permission, from Frank, E. D., Kaufman D., Korman H Schweinburg F Frank, H A. and Fine J Effect of antibiotics on hemodynamics of hypovolemic septic shock. *Am J Physiol* 182, *166 (1955)*

to really low levels suggests that the circulation was not being further damaged by bacterial toxins

Fremont Smith You said he has been in shock 12 hours I do not understand what you mean.

Fine The dog loses a tremendous amount of fluid into the peritoneal cavity during the first few hours and there is marked hemoconcentration

Fremont Smith He has not been in shock according to the usual definition of shock



Hematocrit %	52	64	70	73	76
Plasma vol ml	800	613	527	423	466
Cardiac output ml/min	3690	2870	1,306	1610	2250
A V oxygen diff vol. %	3 5	4 6	9 4	6 2	4 6
Peripheral resistance (p.r u)	028	050	091	081	050

FIGURE 15 Hemodynamics of hypovolemic septic shock (experimental peritonitis) Representative example of effect of prophylactic antibiotic therapy Reprinted, by permission, from Frank, E. D Kaufman, D Korman, H., Schweinburg, F Frank, H A. and Fine, J Effect of antibiotics on hemodynamics of hypovolemic septic shock. *Am J Physiol* 182, 166 (1955)

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Fremont Smith Irrespective of blood pressure?

Fine Irrespective of blood pressure In these terms the data bear out that definition.

Fremont Smith This is only within that definition, which is not the general definition, is it?

Fine I will stand on what I have said that the dog pretreated with antibiotic was in shock

Horvath Hypothermia is a state of shock?

Fine No

Nickerson It would fit the definition you just presented

Fremont Smith It fits in your definition, in a way

Fine It fits the definition in a way To discuss this point would require considerable time so I will answer briefly by saying that in hypothermia the circulation is slow but *not* deficient When we speak of a deficiency it seems to me obvious that we mean deficient with respect to tissue demand The demand is low in hypothermia it is normal in the normothermic animal

Knisely Where is the cardiac output shown?

Fine The cardiac output is high to start with and it falls steadily to a level which in hemorrhagic shock of much shorter duration, usually is inconsistent with survival

Levine Dr Fine in the animals with maintenance of blood pressure and in those in which the blood pressure fell was the oxygen A V difference measured?

Fine Yes

Levine Was it also raised?

Fine Yes it was very high It rose from about 3 up to as many as 12 volumes per cent

Knisely Dr Fine up to now you have been discussing hemorrhagic states and in general I should think the red cells were not agglutinated in straight hemorrhagic shock Do you know whether they were agglutinated?

Fine No we did not look for agglutination

Burch Did the dogs have such a large cardiac output because they were large and frightened?

Fine The cardiac output varies from 3 to 5 liters in the circumstances

Burch Were the dogs large?

Fine Most of them were large dogs

Burch Do you know the cardiac output in a sleeping dog?

Fine No All had an average dose of morphine 2 hours before we started the experiment

Srikantia Were the dogs in clinical shock?

Fine You mean did they have cool extremities? Yes and they had pale cyanotic mucous membranes

Horvath Were the rectal temperatures 105 F?

Fine The rectal temperature was 105°F at one point in one case Whether the fever is high or moderate or low the mucous membranes look as they do in hemorrhagic shock.

Fremont Smith A patient in typhoid vaccine fever with temperature rising to 105°F will look exactly as though he were in shock and his blood pressure will be down, too

Horvath The blood pressure is not down, but the cardiac output is down.

Fine If one blanches the mucous membranes one sees very sluggish return of flow

To me the data given in Figures 13, 14 and 15 signify that a dog can remain in deep hypovolemic shock for many more hours than a dog in hemorrhagic shock, and still survive so long as the circulation remains free from the influence of toxins

Shorr What was this dog under the influence of then?

Fine The dog was under the influence of antibiotic.

Shorr No you injected a toxin or bacteria.

Fine We injected a suspension of bacteria, perhaps with some toxin and whatever else there may be in a fecal suspension

Shorr Largely bacteria let us say

Fine Largely bacteria.

Shorr What was he under the influence of that gave him all of these profound changes? And what was he protected against?

Fine Do you mean the dog that received antibiotic?

Shorr The animal you just referred to

Fine I think he was under the influence of an irritant that produced a severe oligemia

Shorr Any relation to the bacteria?

Fine A fecal suspension is a mixture of dead and living bacteria and their metabolites, split products of fat and protein and carbohydrates cellular debris etc Only bacterial activity is suppressed by the antibiotic The rest of the activity in that suspension is allowed to act That it does act is shown by the fact that a large volume of fluid is lost into the peritoneal cavity and the animal develops oligemic shock

Shorr So he is under the influence of something other than bacteria?

Fine Yes

Shorr Which produces something against which an antibiotic protects Is that right?

Fine No it is not correct to put it that way

Shorr How would you like to put it?

Fine The antibiotic protects against bacterial activity in the injected

suspension. The other properties of the injected substances are able to induce severe hypovolemic shock.

Levine How do you explain the maintenance of the blood pressure in view of those changes?

Fine I do not know except to say that in the early stages plasma is lost, but not red cells. The resulting high blood viscosity tends to increase peripheral resistance. Later when collapse of the circulation from bacterial toxins should be occurring no collapse occurs so that the blood pressure does not fall much more. To the extent that blood pressure falls in response to a state of uncontrolled sepsis, it does not fall in the presence of an effective antibiotic.

Fremont Smith I should like to make one comment here in connection with these two different kinds of shock that is the kind of shock associated with a low blood pressure and the kind of shock that you speak of which does not have to have a low blood pressure It seems to me one essential difference is in respect to what is happening to the central nervous system The kind of shock associated with a low blood pressure exposes the brain to certain difficulties and certain reactions whereas the kind of shock associated with the high blood pressure is presumably delivering to the brain an adequate amount of blood and it seems to me this is one difference to be thought of There are probably other differences too

Fine I do not deny that these other things you describe may be occurring but the main point about the shock we are dealing with here is that the rate of flow through the tissues is grossly deficient whatever the blood pressure

Fremont Smith Through the brain.

Fine I should say everywhere We have evidence that flow through the brain is better than in other parts of the body But in terms of normal values, flow is deficient everywhere (16)

Kinsell There is one situation in which human beings receive feces into the circulation. Lushbaugh and Steiner (17 20) have shown that meconium sometimes gets into the maternal circulation at the time of birth, and that this material sometimes plugs up great numbers of the tips of pulmonary arteries thereby reducing the flow of blood through the lungs and forcibly reducing the filling of the left heart This is the direct cause of one type of maternal circulatory shock and death Do you think that perhaps partial plugging of pulmonary a[illegible] feces contributes to the low cardiac [illegible] a

Fine No in this case we inject a[illegible] sion into the cavity Meconium is a sterile substa[illegible] the mothe veins

Dobson It seems to me that there is a tendency here to believe that there cannot be a low cardiac output and blood flow with a normal blood pressure I should like to say that in Dr Warner s and my experiments with burn shock (21 24) we get immediate reductions in cardiac output These pronounced reductions in total body blood flow have been noted within 10 minutes and the flow may fall to as low as one seventh of the preburn cardiac output with essentially normal blood pressure Incidentally, blood pressure usually stays normal for several hours in spite of the reduced output from the heart So it looks as though we may be getting into difficulty with a definition for shock In burns there is a situation in which the general circulation, as measured by the cardiac output and liver blood flow is probably insufficient for the body needs On the other hand in shock accompanied by hypothermia there may be a greatly reduced cardiac output, but the reduced need occasioned by the hypothermic state may well create a situation in which this reduced blood flow is not a deficient one.

Bradley You mean every time you stand up cardiac output falls?

Dobson I did not say that a fall in cardiac output was shock I said I think maybe we need another definition or maybe we just ought to stop using the term.

Fremont Smith We should say shock with respect to and say how we are defining it. Dr Fine does that When he says shock, he defines the term as deficient peripheral circulation regardless of anything else Thus is the only criterion he uses Other people use other criteria. It seems to me it is very important that we say what criterion we are using When we say hemorrhagic shock, we are not measuring it in terms of peripheral circulation, but of blood pressure at a certain level

Fine I am dealing fundamentally with peripheral flow There are situations in which the blood pressure does not fall significantly yet deficient flow is present This is not the case in hemorrhagic shock but it is true of tourniquet shock and as Dr Dobson says, of burn shock.

Tissue damage occurs in all of these conditions But the tissue damage due to deficient flow can be tolerated for much longer if toxins can be excluded The absence of toxins is what protected these dogs from death They recovered from hypovolemic shock as a result of reabsorption of the fluid in the peritoneal cavity If they could not reabsorb this fluid and none was available from other sources they would of course eventually die

Burch Do you have any information about the veins?

Fine No

Burch Do you know if venous tone changes?

Fine No

Burch You say the circulation is deficient but actually all you know is that blood supply was less

Fine In terms of the fall in cardiac output i e to 25 per cent of normal I consider the peripheral flow severely deficient This is shown also by the fact that the A V difference remains very high in these animals

Burch This returns to the hypoxic state mentioned by Dr Shorr There was less blood supply but have you actually shown there was an inadequate amount of blood reaching the vital structures?

Fine I offer the suggestion that if the tissues require 5 liters but are getting only 1 liter and the A V difference is very high the tissues are suffering for lack of sufficient blood

Green Haven t we gone through the complete cycle which we started in the conference the first year by setting up states of shock and one was a state in which the mean arterial pressure was normal but the cardiac output was below a level which would sustain a posthemorrhage animal? Do you remember?

Fine Yes

Burton At the first conference we decided that there are many ways of characterizing shock with short words and that we should use them for example, hypotensive and normotensive shock By use of simple words like this then we could remove some of these difficulties

Shorr I recall Dr K J Franklin s asking at the beginning of the first conference 'Would it be possible for you to define shock before we start? I felt that the necessity to provide definitions on which we could all agree could easily consume the entire session!

Fine Giving the antibiotic after inducing shock was of no value in these dogs There were similar results from this difference in timing in hemorrhagic shock We tried to analyze the way in which timing the antibiotic affects the result in another way as follows

Normal and shocked rabbits were exposed to their respective M L.D /100 doses of *E. Coli* toxin as I have already described Antibiotic was given parenterally in advance in four ways to groups of ten animals each In the first group it was given 4 hours before the toxin in the second at the same time in a separate vein and in the third at the same time in the same vein the toxin and antibiotic having been mixed 4 hours beforehand and kept at 4 C for 4 hours before injection Table XXIII shows a 50 per cent survival rate even with doses up to twice the M L.D /100 in both the normal and shocked rabbits given antibiotic with or prior to the toxin *The antibiotic did not protect* against 3 times the M L.D /100

Table XXIII shows that in the fourth group of rabbits the non

absorbable antibiotics bacitracin and polymyxin, given orally for several days in advance of the challenge dose of toxin and also shortly beforehand, acted as well as those given parenterally This action can be said to be related exclusively to an effect upon the intraintestinal flora

Shorr Can you say absolutely nonabsorbable?

Fine I think so According to the literature, they are the only ones that are nonabsorbable About 3 per cent of neomycin does get absorbed

Fremont Smith It might be absorbable in a shocked animal rather than in a normal animal.

Fine Except for some slowing of absorption caused by shock, there is no reason to expect the effect to differ in the normal and shocked animal.

To a fifth group of dogs not shown in Table XXIII antibiotics were given 30 60 or 90 minutes after the toxin There was no protection whatsoever

The difference in the result from differences in timing the antibiotic is a tantalizing puzzle The results might be interpreted as follows The fact that the nonabsorbable antibiotics are equally effective indicates that bacterial invasion from the intestine is a continuous process and is involved in the death from an M.L.D of toxin In the healthy state these bacteria are being continuously destroyed and their endotoxins neutralized. If an M L.D of toxin is given, it temporarily impairs the bacterial defense mechanisms, so that any more toxin released soon thereafter from invading bacteria cannot be neutralized and acts to paralyze some vital function, with death resulting in 8 to 24 hours With an effective antibiotic already present, no more toxin can be released. But if a short interval after giving the toxin is allowed before giving the antibiotic, an additional dose will be generated This dose, however small will be fatal if there is no defense whatever available at the time. This view was supported by the following experiments, in which no antibiotic was given One hour after a nonlethal dose of toxin was given, a second dose was administered This was fatal in most cases even when this second dose was as little as 1/128 of the first dose and the sum of the two doses much less than one M L.D This second dose however did not kill if an antibiotic was given along with the first dose. Antibiotic was not necessary to protect against the second dose if the second dose was given after 4 hours instead of after 1 hour After 4 hours it proved quite harmless These observations are in line with A. L. Braude s observations* that bacterial toxin destroys leukocytes

*Unpublished.

TABLE XXIII

Effect of Antibiotic Therapy on the Survival Rate of Normal and Shocked Rabbits Subjected to Their Respective MLDs (100) of Bacterial Toxin or Multiples Thereof

No. Exp.	Antibiotic	Toxin: Dosage	Toxin: Time Relative to Antibiotic Therapy	Normal: Number of Rabbits	Normal: Survival (per cent)	Shock: Number of Rabbits	Shock: Survival (per cent)
	None	1 MLD		20	0	20	0
I	Penicillin and streptomycin	1 MLD	4 hours after	10	60	20	45
	Penicillin and streptomycin	1½ MLD	4 hours after	10	50	20	50
	Penicillin and streptomycin	2 MLD	4 hours after	10	50	10	50
II	Penicillin and streptomycin	1 MLD	Simultaneously	20	55	20	45
	Penicillin and streptomycin	1½ MLD	Simultaneously	10	60	20	45
	Penicillin and streptomycin	2 MLD	Simultaneously	20	60	10	50
	Penicillin and streptomycin	3 MLD	Simultaneously	10	0	—	
III	Penicillin and streptomycin	1 MLD	Simultaneously	20	65	10	40
	Penicillin and streptomycin	1½ MLD	Simultaneously	20	60	10	60
	Penicillin and streptomycin	2 MLD	Simultaneously	20	60	10	50
IV	Bacitracin and polymyxin or neomycin	1 MLD	Immediately after 4th dose or 6 hours later in 2 hour shocked dog	10	50	15	60
	Bacitracin and polymyxin or neomycin	1½ MLD	Immediately after 4th dose or 6 hours later in 2 hour shocked dog	10	50	15	33
	Bacitracin and polymyxin or neomycin	2 MLD	Immediately after 4th dose or 6 hours later in 2 hour shocked dog	10	50	10	40

TABLE XXIV

Effect of Type of Therapy for Tourniquet Shock in Rats on Survival Rate and on Recovery of Bacteria from Injured Muscle

Group	Type of Therapy	No. of Experiments	Survivors Over 48 Hours (per cent)	No. of Animals Cultured	Percentage of Cultures of Tourniqueted Legs Positive for *Clostridium welchii*
I	None	37	0	10	90
II A	Fluid, on removal of tourniquets	15	73	15	66
II B	Fluid started 6 hours after removal of tourniquets	5	80	5	100
II C	Fluid started 12 hours after removal of tourniquets	8	62	3	100
II D	Fluid started 18 hours after removal of tourniquets	9	66		
			Average 70		
III	Aureomycin prior to application and after removal of tourniquets	7	14	7	0
IV A	Same as III plus fluid on removal of tourniquets	14	86	12	16
IV B	Same as III plus fluid started 6 hours after removal of tourniquets	7	100	7	28
IV C	Same as III plus fluid started 12 hours after removal of tourniquets	9	89	3	0
IV D	Same as III plus fluid started 18 hours after removal of tourniquets	6	84	5	20
			Average 88		

No other bacteria found

Those which disappear are replaced by others when the toxin has been removed from the circulation by the reticuloendothelial system This occurs within 4 hours

Much of what is occurring here will be shown eventually to apply to the state of irreversibility to transfusion in shock.

The validity of much of the speculation I have here indulged in depends upon the magnitude of absorption from the gut either of toxin or of bacteria That bacteria, in fact get in was directly demonstrated by the following observations Rats were subjected to mild muscle trauma inflicted by a few hammer blows upon the thigh Others were subjected to this trauma and then put into reversible hemorrhagic shock, i e., 2 hours of shock followed by transfusion. Rats like dogs and rabbits survive if transfused after 2 hours of hemorrhagic shock

Both groups of rats as well as normal rats received a dose of *Streptococcus viridans* directly into the gut No bacteria were found anywhere in any of the normal rats or those with muscle trauma But some were found in the shocked rats with muscle trauma Therefore, bacteria are absorbed from the gut into the circulation, and they thrive in the blood and traumatized muscle of the shocked rat because the bactericidal mechanisms are impaired

Further data on this point are the following observations on tourniquet shock in the rat Table XXIV shows that normal rats in shock after exposure to tourniquets for 4½ hours die Most of them at death show *Clostridium welchii* in the muscles of the legs and nowhere else These muscles are sterile before the tourniquets are removed but within 2 to 3 hours after removing them, *C welchii* are found and the incidence of positive cultures increases steadily until death I suggest that the *C welchii* come from the gut

If such rats are treated with water orally and saline intraperitoneally most of them survive even if the fluid is first administered as late as 18 hours after removal of the tourniquets

Haist For how long were the tourniquets kept in place?

Fine For 4½ hours

Haist Quite different results are obtained from 1½ hour periods of tourniquet application in rats than from 10-hour application periods

Fine Of course I have no doubt of that

Haist Certain fluids which aid survival in the 4½ or 5 hour clamped animals are not as effective in animals clamped for longer periods

Fine We have had a similar experience in dogs Saline therapy or plasma was effective for shock in dogs produced by a 1½ hour tourniquet But plasma even in huge volume failed in shock following 8 to 10-hour tourniquets because the dog s muscles become heavily infected

Haist It looks as if with the short period of clamping there is a straight fluid loss factor

Fine Yes. In the rat it is primarily dehydration shock because simple saline therapy is effective The presence of *C welchii* indicates that either they are not very virulent or that the animal s resistance to them is not seriously impaired.

In the next group of experiments the rats were not given fluid but aureomycin was given prior to the application of the tourniquets It did no good, in spite of the fact that *C welchii* were prevented from surviving in the muscles In other words the fluid deficit is the primary need and the bacterial factor is of minor consequence

If aureomycin and fluid therapy are combined, there is a perceptible difference in the survival rate. Instead of 70 per cent with fluid therapy alone we obtained an 88 per cent survival rate. Giving the aureomycin perhaps adds a little protection

Shorr That is protection in that small series of animals

Furchgott With nine animals that is not a significant difference.

Fine Nine is not a significant number But if we lump all the rats receiving fluid therapy alone, there are thirty seven with a 70 per cent mortality rate, and thirty six receiving aureomycin plus fluid therapy with an 88 per cent survival rate. This is a real though minor difference and is caused by suppression of the *C welchii*

The main point is that this kind of injury is primarily dehydration shock and bacterial element is of small, if any consequence

Table XXV shows that in hemorrhagic shock in the rat antibiotics given in advance protect as they do in the dog although not so effectively Tolerance to hypotension is increased with aureomycin The survival time of nonsurvivors is prolonged and the mortality rate reduced considerably Penicillin is not useful in the rat, but it is in the dog

It is commonly agreed that the rat s bactericidal potential is greater than the dog s One of the reasons may lie in Dr Pillemer s data on their relative properdin titers Even so the rat s bactericidal mechanism is injured by irreversible shock, whether resulting from tourniquets or hemorrhage. Thus a normal rat will readily destroy certain intravenously injected bacteria so will a rat with mild muscle trauma. A rat in tourniquet shock will not do so well if challenged after having been in shock for several hours As many as 33 per cent of such rats die in spite of adequate fluid therapy The same is true of rats in hemorrhagic shock for 2 hours The mortality of dogs in hemorrhagic shock challenged by bacteria is 100 per cent

TABLE XXX

Tolerance of Rats Receiving Antibiotics to Hemorrhagic Shock (data are average values)

Group	Antibiotic	No. of Rats	Max. Bleeding Vol (ml/100 gm body wt)	Duration of Hypo-tension (hr)	Survival Rate (per cent)	Survival Time of Non survivors (hr)
Control	No antibiotic	30	3 3	3¼	13	4¼
I	Aureomycin	20	3 2	7½	40	12¾
II	Neomycin	14	2 5	4½	50	12
III	Penicillin	8	2 6	5 0	12	3½

Haist Dr Fine do you grant that tourniquet shock is shock resulting from fluid loss?

Fine The 4½-hour tourniquet injury produces a plasma filtrate loss

Haist If you grant that we have then a reduction in circulating blood volume and the general criteria for shock Yet these animals are not protected by pretreatment with aureomycin Must you not grant then, that the bacterial factor while it may enhance the course of development of shock is not fundamental to that course of development because these animals die despite the aureomycin?

Fine Which ones?

Haist The ones shocked for 5 hours

Fine If they are not given fluid of course they die

Haist If they are given aureomycin they still will die

Fine If they are not given fluid they will There is no reason to expect aureomycin to be a substitute for fluid

Haist What were your survival times in those animals that were shocked by 1½ hour tourniquet application

Fine They survive permanently if given fluid without fluid they are dead within 18 hours

Haist What was the mean survival time?

Fine I do not know perhaps 24 hours

Haist They survive for quite a long while

Fine Yes they do Many are alive up to 48 hours.

Haist One can have nearly all the criteria of shock that are obtained following hemorrhage and yet these animals die despite aureomycin Therefore, I submit that the irreversibility that develops, irreversibility which will ultimately lead to death of the organism, cannot be the result solely of the infective factor as you postulate

Fine I do not understand your point. The loss from the circulation in tourniquet shock is primarily a loss of some constituents of plasma i e water some electrolytes and probably some protein whereas in hemorrhagic shock the loss is whole blood Hence the injury to the tissues is greater in the case of whole blood loss because less oxygen is being delivered

Haist The fact of the matter is that these animals died

Fine They die because of the absence of fluid I do not know what point you are making They do not die if they are given fluid.

Shorr Dr Haist s point is that although the animals do not have bacteria to combat, they deteriorate in a state of circulatory failure. Is that correct?

Haist Yes and I should also like to comment on the fluid therapy If these animals are given saline as a single dose, a quite different effect on survival time will be obtained then if it were given as a continuous infusion over a period of 24 hours In rats with limb ischemia for a period of 5 hours if a single injection of saline or an injection of saline over a period of 2 hours is given at a rapid rate there may be about 17 per cent of the animals surviving whereas if given slowly over a 24-hour period there may be 40 per cent survival

Fine We gave the fluid therapy in increments up to 18 to 24 hours Dr Hardy (14) worked with Dr DeBakey and his data should be mentioned in connection with Dr Pillemer s data on the difference in the properdin titer of dogs in different parts of this country This will be described by Dr DeBakey later in the discussion

Kinsely Dr Fine has shown that bacteria have an effect upon survival and upon shock So far I am not convinced that he has shown that bacteria are essential to produce either death or shock I should like to ask a totally unfair question There are places where animals such as monkeys and mice and guinea pigs are raised completely bacteria free. Dr Fine, do you believe it is possible to put such animals in the shock state?

Fine Dr Zweifach has done this and will discuss it later

Shorr May I now turn to another aspect of this problem which would

suggest that the description of a substance as an antibiotic may not indicate all of its actions and that when inferences are drawn as to the influence of such an agent upon an animal we should be prepared for a possibly wider spectrum of action than is implied by this term. However, if we try to define the nature of antibiotic action we must admit that, unless it works in a mysterious fashion which defies elucidation, it very probably affects the metabolic activities of the bacterial cell and hence may at the same time act upon the host at the cellular level. With this in mind I have asked Dr. Baez to discuss some of the work being done in our laboratory at the present time.

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AN INQUIRY INTO THE MECHANISM OF THE PROTECTION AFFORDED BY AUREOMYCIN AGAINST SHOCK*

SILVIO BAEZ†
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OUR REASON FOR STUDYING the protection afforded against shock by a variety of agents such as antibiotics, nerve blocking drugs etc. is to clarify the relationship of the hepatic ferritin systems to such protection. The studies with aureomycin described herein were carried out in our laboratory over the past several years

Hemorrhagic shock was induced by a method which consists essentially of graded bleeding controlled by a self infusion apparatus as previously described (1) Blood pressure was maintained at or above 70 mm. Hg for the first hour at 50 mm. Hg during the following hour and at 30 mm Hg for the remaining 2 hours The amount of blood shed duration of the maximal bleeding and amount of blood uptake during the experiment were noted. Blood which had not been taken up spontaneously at the end of the 4-hour period was gradually force infused

At the termination of the experiment the neck wound was closed and the animal returned to its cage Animals still alive after 24 hours were recorded as surviving

Fremont Smith What animals were used?

Baez. Female rats of the Wistar strain weighing 120 to 150 grams

Fremont Smith Were they anesthetized?

Baez Yes They were given 2 5 mg of sodium pentobarbital intramuscularly

*Aided by research grants from the National Institutes of Health, U S Public Health Service (Grant H 79) the Josiah Macy Jr Foundation the Research and Development Division, Office of the Surgeon General, Department of the Army (Contract No DA-49-007 MD-388) the Armour Laboratories and the Postley Fund. The skilled assistance of Anna Holmquest, Iris Forbes, and Carla Rand in carrying out these studies is gratefully acknowledged

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Shorr The blood pressure is maintained at these levels by a reservoir system

Baez During the drastic hypotensive period (30 mm. Hg) the animal's cardiovascular system was in free communication with the blood reservoir The rat shed blood to a maximal level and, after a lapse of time, started to take up blood from the reservoir

Traumatic shock experiments were carried out by the method of Noble and Collip (2) in unanesthetized rats using 700 revolutions of the drum at 44 rpm

When we started this series of experiments there was no information in the literature on the protective dose of aureomycin for the rat in drum shock We therefore used amounts comparable to those which had been shown to afford protection against hemorrhagic shock in the dog Table XXVI summarizes the data obtained with 15 mg or *100 mg of oral aureomycin per 100 gm. body weight daily for 4* days with the same dose given by gavage on the fifth day 2 hours before drumming The survival rates were 33 per cent in the controls and 50 per cent or 51 per cent respectively in those receiving 15 mg or 100 mg of aureomycin Thus only a modest 17 per cent to 18 per cent improvement in the survival from drum trauma seemed to be afforded by these two dosage levels of the antibiotic

Subsequently we noted that György *et al* (3) had observed that the

TABLE XXVI

Effect of Oral Aureomycin at Two Dosage Levels*
On the Outcome of Drum Trauma in the Rat

Aureomycin	None	15 mg/100 gm	100 mg/100 gm
Number of rats	126	121	49
Number of rotations	700	700	700
Number surviving at 24 hours	41	61	25
Per cent survival at 24 hours	33	50	51

*Aureomycin 15 mg and 100 mg in food daily for 4 days and 15 mg and 100 mg respectively by gavage 2 hours before drum trauma

continued administration of aureomycin to rats sterilizes the intestinal tract for only a short period of time, the bacteria becoming resistant to the drug and returning within 2 weeks In view of this report we repeated the drum trauma experiments at the end of 3 weeks of feeding aureomycin Table XXVII presents the results of this experiment, in which rats were fed 15 mg of aureomycin daily for 3 weeks and 15 mg by gavage 2 hours before drumming The survival was 49 per cent in the aureomycin group as compared with 22 per cent in the controls The increased survival in the treated group suggests that aureomycin might exert effects other than its well known antibiotic action

Nickerson Were the controls in this experiment given an equal volume of saline or water by gavage?

Baez Yes

Nickerson Was this given to them 2 hours before the shock procedure?

Baez Yes, they were given the same volume of water as the experimental group

Green Is there significance in the two sets of controls? For instance in one group you have 33 per cent and in the other group 22 per cent.

Baez There is considerable variation in survival between individual experiments For example, the 126 controls in Table XXVI represent 15 groups in which the survival was by no means 33 per cent in each group We have attempted to overcome this variability by increasing

TABLE XXVII

Effect of Chronic Feeding of Aureomycin* on the Outcome of Drum Trauma in the Rat

Aureomycin	None	15 mg/100 gm
Number of rats	36	39
Number of rotations	700	700
Number surviving at 24 hours	8	19
Per cent survival at 24 hours	22	49

Aureomycin 15 mg given in food daily for 3 weeks and 15 mg by gavage 2 hours before drum trauma.

the numbers of rats in each of our series and by the consistent use of a parallel control group for each treated group We believe that this prevents environmental factors and seasonal variations from unduly influencing the results

Fine May I ask what the rats are dying from? You say drum trauma. Is it drum shock? I was asked the same question about our dogs Perhaps you can tell us about these rats

Baez In answer to your question as to what the rats are dying from I shall describe the general appearance of these animals as they come out of the drum The control rats are in very poor condition. They look stunned with cyanotic paws and snoot They are hyperpneic and remain very quiet in their cages If the carotid artery is cannulated under local procaine the blood pressure is found to range from 40 mm Hg to 80 mm Hg and to decline steadily the majority die within 3 hours Under direct microscopic observation the terminal vascular beds of the mesoappendix and mesentery as well as of the wall of the alimentary canal show striking functional deterioration with decreased sensitivity to topical epinephrine or arterenol, absence of spontaneous vasomotion, sluggish blood flow and numerous foci of petechial hemorrhage These vascular derangements are comparable to those observed in the irreversible stage of hemorrhagic shock The microscopic appearance of the vasculature of the alimentary canal after drum trauma has not been described previously In addition to the over all congestion of the wall of the gut numerous enlarged venules containing stagnant blood and zones of constriction may be seen The large numbers of these pockets of sequestered blood give the impression that the animal has been bleeding into its own gut

Fine You do not know what the blood volume is?

Baez No

Zweifach Noble and Collip (2) and co-workers have published work on the blood findings in these animals as well as the gut pathology liver congestion, adrenal engorgement etc On the basis of Dr Fine's own criteria of shock these rats are in a state of shock

Fine I agree Didn't they treat them with fluid?

Zweifach Drum shock cannot be combatted successfully by fluid therapy alone either in the form of pretreatment or after the induction of shock

Baez. Table XXVIII gives data on two sets of experiments in which we gave aureomycin intravenously in a minimal dose 100 μg/100 gm of body weight This exceedingly small dose was suggested by *in vitro* experiments in which 10 μg of aureomycin/gm of tissue were sufficient to block the liver's anaerobic release of ferritin I shall discuss these

TABLE XXVIII

Effect of Intravenous Pretreatment with Aureomycin*
On the Outcome of Drum Trauma in the Rat

Treatment	Saline	100 μg aureomycin/100 gm body wt	
Time of injection†	−60 to −120 min	−60 min	−120 min
Number of rats	47	29	47
Number of rotations	700	700	700
Number surviving at 24 hr	22	13	36
Per cent survival at 24 hr	47	45	77

*Aureomycin was given by the tail vein.
†Minutes before rotation in drum.

in vitro studies later To return to the traumatic experiment, one group of animals was drummed at 60 minutes and the other at 120 minutes after the injection The controls received the same volume of saline intravenously It may be seen that increased survival (30 per cent above the controls) appears only in the group drummed 2 hours after the injection of the antibiotic

Shorr I think the group might be interested in knowing why we selected 120 minutes

Baez In our first I V drum trauma experiments the dose of aureomycin was administered 1 hour before drumming The choice of this time was based on the protective effect obtained in hemorrhagic shock with the same dose and time interval We were however discouraged with the results very little protection was found against drum trauma We reasoned therefore that this discrepancy in protection might be related to a time factor the drastic hemorrhagic hypotension might be assumed to become maximally harmful only after it had been maintained for

several hours while in drum trauma the entire stress was imposed within the 15 minute period of the rotation in the drum

Horvath Since you are explaining why you used aureomycin at that particular time, could you explain something else? Dr Fine has insisted and rightly so that their use of 24 hours survival is not necessarily a good criterion that survival should be for an indefinite period of time Would it be possible from the data you have to include also the survivals greater than 24 hours, i e., to state whether these animals survive 24 or did they survive 48 72 100-odd hours?

Baez I am glad you brought that up Dr Horvath because to me the term permanent survival implies keeping the animals for their whole normal life span In our laboratory we tabulate survival results at 24 hours This does not necessarily mean that we sacrifice the animals at the time, and usually they are kept for a week before being sacrificed In our experience, deaths after 24 hours occur in a larger number of the remaining control animals than in the treated group

Shorr If the time interval were prolonged to more than 24 hours the mortality in the controls would be correspondingly greater

Baez I believe that the selection of time 24 48 72 hours or what ever is an arbitrary criterion

Shorr I think that this is the situation One group defines recovery as permanent and the other group feels that recovery at the end of 24 hours deals with the shock syndrome *per se* and that other causes of death may enter into the later deterioration We understand that Dr Fine s data are based on longer survival However we are content at this time to record 24-hour survival if we actually kept our animals longer as we often have done there would not be a large discrepancy between those results and the ones we report

Fremont Smith Permanent is a meaningless term too because none of the animals survives permanently You would have to say into old age and indefinitely meaning unmeasured or until we got tired of the experiment and killed them.

Zweifach Our experience with this type of trauma has been that rats which survive for more than 24 hours usually live indefinitely In a series of 24 hour survivors encountered over a period of several months 95 out of a 100 of these animals lived for at least 30 days

Fine Ten to 20 per cent of our dogs in hemorrhagic shock pretreated with antibiotics and with a good response to transfusion survived for only 36 hours or less If they survived 48 hours they survived indefinitely

Srikantia At the end of 36 hours had these dogs come out of shock?

Fine They come out of shock after the transfusion

Srikantia After they come out of shock and die of other factors later on would you attribute the death to shock?

Fine We attribute the death to a septic factor which takes hold because the impaired bactericidal mechanisms are still below par The septic factor may kill as a result of recurrent shock, but we have no data on this

Shorr We cannot at this juncture attempt to resolve these differences We can only express them and I think that has been done quite clearly

Birch How many of your rats survived according to Dr Fine s criteria?

Shorr Most of them did

Green Shouldn t both Dr Fine s and Dr Baez s data be subjected to the chi square analysis and the data given to us on the charts? I should like to hear from Dr Burton on this subject because he made an analysis a while ago

Burton I do not wish to press this in any hostile way but I do feel that few people understand how easy it is and how little time it takes to learn with a statistician how to do a chi squared test on a table like Table XXVI It can probably be mastered in an afternoon as it is only an elementary manipulation of the figures In this way it is possible to determine whether these data are statistically valid or not. Unless we do this, a lot of time is wasted because the data are borderline and not significant therefore perhaps they should not be emphasized

I have roughly applied the chi squared test to the above data and I should say the differences were far from significant but I am not sure whether or not the speaker was emphasizing that they were significant.

Baez In the oral feeding experiments only modest protection was offered against drum trauma. The differences in survival between the controls and the treated rats in Tables XXVI XXVII and XXVIII would according to statistical theory (4) not be likely to occur by chance since they are more than 2½ times the respective standard errors of the differences

Horvath It would be nice to see the statement in the tables that there is significance without requiring us to make the deduction from the raw data.

Baez. Table XXIX summarizes the data obtained in hemorrhagic shock with a single dose of 100 μg/100 gm. body weight administered by the portal vein in order to achieve rapid exposure of the hepatic cells to the antibiotic Pretreatment with this minimal dose of aureomycin increased the survival after our standardized graded hemorrhagic procedure to 68 per cent, i e. 40 per cent above the control

TABLE XXIX

Effect of Intraportal Aureomycin* On the Outcome of Hemorrhagic Shock in the Rat

	Controls	Aureomycin
Injected into portal vein	0 2 ml saline	100 μg/100 gm b wt
Number of animals	18	19
Maximum blood lost† (per cent body weight)		
Average	3.2	3 4
Range	2.2 to 3.8	2 4 to 4 0
Duration of maximum bleeding (min)		
Average	28	45
Range	15 to 90	15 to 105
Uptake of blood** (per cent body weight)		
Average	0 5	0 4
Range	0 0 to 1 5	0 0 to 0 9
Per cent survival at 24 hours	28	68

*Aureomycin injected 60 minutes before hemorrhage
†Total blood loss required to bring blood pressure to 30 mm Hg
**Amount of blood spontaneously taken up from reservoir to maintain blood pressure at 30 mm Hg for 2 hours.

group which received saline by the same route of administration The average duration of the maximal bleeding was 28 minutes in the control group as compared to 45 minutes in the aureomycin group Although the differences in the amount of blood shed (0 2 per cent of the body weight) and in the uptake from the reservoir (0 1 per cent of the body weight) appear small they represent important volumes for the rat

The data are graphically presented in Figure 16 which shows that (*a*) equal or slightly larger blood loss was achieved by the treated rats and therefore a larger residual circulating blood volume seems not to be responsible for the favorable outcome in this group and (*b*) the blood uptake from the reservoir which occurs spontaneously in order to maintain the blood pressure at 30 mm. Hg was considerably delayed in the experimental group possibly reflecting a more adequate adjustment of the cardiovascular system at the drastic hypotensive levels

Fremont Smith : How much fluid went into the portal vein with the aureomycin, the same amount of fluid as in the controls?

Baez The same amount The pattern of blood flow observed in the terminal vascular bed in the mesoappendix confirmed the impression of a better vascular status in the treated rats which had been inferred from

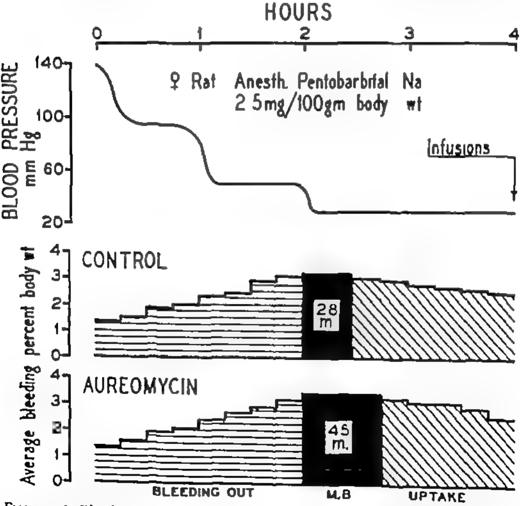

FIGURE 16. Bleeding pattern in aureomycin treated rats and their controls M.B is average maximal blood loss and m is average duration (*in minutes*) of the maximal blood loss.

the delay in spontaneous uptake Such direct microscopic observations were carried out in a small series of control and experimental rats Aureomycin or saline was injected into the tail vein The exposure of the cecum constituted an additional trauma which did not appear to alter the final outcome

During the early stage of the hemorrhagic procedure there was no noticeable difference between the control and the aureomycin treated rats in their vascular adjustment The compensatory phase characterized by tonic arterial and arteriolar vasoconstriction enhanced vasomotion, and increased epinephrine sensitivity of the metarterioles and precapillary sphincters was equally present in both groups At maximal compensatory readjustment, the blood flow was mainly effected through the preferential channels and A V shunts After a variable period of time a pattern of behavior developed in the control group conducive to a rapid deterioration of the integrity of the vascular bed and peripheral vascular collapse The initially insidious development of these vascular changes became pronounced after about one hour of drastic hypotension (30 mm Hg) with characteristics distinctly different from those in the treated rats The sensitivity to epinephrine declined vasomotion of metarterioles and precapillaries rapidly ceased and the large arteries and arterioles became less constricted or atonic One of the most striking features was the occurrence of numerous foci of petechial hemorrhage on the venular side of the true capillaries and in the endothelial venules indicative of an alteration suffered by the vessel walls These petechial hemorrhages became more numerous with the increasing spontaneous uptake of blood and were maximal with the reinfusion of the remaining blood at the end of the experiment In contrast to the vascular deterioration seen in the controls with the same degree and duration of hypotension, the terminal vascular bed of the animals pretreated with aureomycin continued to exhibit compensatory types of reactions

Fremont Smith Hyperreactivity was maintained much longer in the aureomycin-treated animals?

Baez Yes this compensatory type of vascular behavior was well maintained to the time when the experiment was terminated In the control animals the muscular collecting venules of about 80 to 120 μ in diameter usually had an atonic distention of the wall and became engorged with a sluggish blood flow by the end of the first hour of drastic hypotension The tonicity of the wall never recovered in the majority of the dying rats in spite of transfusion of all the shed blood In contrast to this the muscular collecting venules in the aureomycin treated rats showed an unusual tonic contraction which persisted throughout the hemorrhagic procedure

Fremont Smith The vasoconstriction occurs in the mesoappendix under aureomycin?

Baez. Yes in the mesoappendix With transfusion the venule diameter gradually returned to normal and an effective *vis a tergo* with rapid flow of blood was fully restored Venular petechial hemorrhage was rarely observed in the protected animals

Levine Does aureomycin given to an animal whose appendix you have exposed lead to any change of the circulation in the mesoappendix in the absence of shock?

Baez Yes depending on the dose That was one of the reasons why we tried to find a minimal dose which would still be protective Intravenous injection of from 100 to 500μg of aureomycin/100 gm body weight induces a hyperreactivity to topical epinephrine in the terminal vascular bed which lasts from 30 to 70 minutes, doses of less than 50 μg are vasoinert.

Srikantia There is no change in circulation *per se* · The only effect is that the vessels become sensitized to the effect of epinephrine.

Fremont Smith In other words, if no topical epinephrine is given there is no observable change in circulation on aureomycin injection

Frank A large dose of aureomycin given intraportally may kill a normal dog

Baez. We are reporting on rats in all these experiments I only wanted to point out that aureomycin *per se* hypersensitizes the small muscular vessels of the mesoappendix to the topical stimulus of epinephrine However with 100 μg dose we used this effect had disappeared before the stress procedure was begun.

Since our previous studies (5 6) had shown that derangement of the hepatic ferritin oxidation-reduction systems accompanies the vascular deterioration of shock, it became of interest at this point to ascertain the status of the ferritin systems in the liver of the rats pretreated with aureomycin as compared with their controls In another small series therefore the liver was removed at the end of the 4 hour hemorrhagic experiment for *in vitro* studies Heparinized blood obtained at this stage was also bio-assayed (7) The scattergram in Figure 17 presents the results of these experiments The majority of the control livers had completely lost both their ability to restrict ferritin release to anaerobiosis and their capacity aerobically to inactivate added ferritin In the remainder there was impairment of these mechanisms On the other hand the hepatic ferritin oxidation reduction systems in the aureomycin injected rats were still intact at the end of the hemorrhagic procedure Bio-assays of blood obtained from the aureomycin treated rats showed strong VEM (vasoexcitor material) activity while the

		Blood 0 3 ml	Liver slice incubation $O_2 \times 60$ minutes	Plus ferritin in O_2 $\times$ 120 minutes
V E M	Strong	○ ○ ○ ○ ○		
	Mod			
	Mild			
	Neutral	●	○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
V D M	Mild	●	●	●
	Mod	●	●	●
	Strong	● ● ●	● ● ● ●	● ● ● ●
			○ = Aureomycin	● = Controls

FIGURE 17 Effect of pretreatment with aureomycin (100 μg/100 gm. body weight given intraportally 60 minutes before the hemorrhagic procedure) on hepatorenal vasoactive factors in blood and liver after 4 hours of hemorrhagic hypotension (at or above 70 mm. Hg for the first hour at 50 mm. Hg for the second hour and at 30 mm Hg for the following 2 hours)

blood samples from the control group usually contained VDM (vasodepressor material)

Fine I do not quite understand the difference between the middle column and the right hand column

Shorr The livers were excised at the end of the shock experiments sliced, and incubated aerobically in Ringer phosphate Those from shocked controls released vasoactive ferritin aerobically as if the Pasteur mechanism were abolished whereas livers from the aureomycin treated rats did not release ferritin under these conditions they behaved like the livers of normal animals

The second challenge to which we exposed these livers was a test of their ability to inactivate a known amount of crystalline ferritin Fresh slices from these livers were incubated aerobically in Ringer phosphate

containing ferritin. At the end of 2 hours the medium was assayed by the rat test. A vasodepressor reaction indicates that the liver has lost its inactivation capacity. Dr. Mazur has demonstrated that this inactivation is actually an oxidation of the vasoactive, sulfhydryl ferrous form of ferritin to the vasoinert, disulfide ferric state (8). Under the conditions of our experiments normal liver slices are able to inactivate this amount of added ferritin completely. Livers from the control shocked rats had generally lost this capacity while those from the aureomycin treated rats completely inactivated the ferritin.

We have used antiferritin serum as a control in experiments of this nature. The antiserum specifically inactivates ferritin as assayed by the rat mesoappendix method.

Pillemer: Have you tried any analogs of aureomycin to see if you can obtain this effect?

Shorr: We have obtained a few of these compounds but at present we have too few experiments to report.

Fine: Have you tried any antibiotic other than aureomycin?

Shorr: We have worked only with aureomycin.

Baez: I should now like to describe our *in vitro* studies with normal rat liver and I shall first explain the method. The rat is killed by a blow on the head, the liver is removed, placed in a chilled Petri dish and sliced immediately as for tissue microrespiratory studies. The slices (1 gm.) are incubated in oxygen for 10 minutes at 37.5°C. with 10 µg. of aureomycin freshly dissolved in 5 ml. of Ringer phosphate medium. At the end of this time the Ringer phosphate aureomycin medium is discarded and the slices are washed twice with fresh Ringer phosphate. For anaerobic ferritin formation, fresh Ringer phosphate is added in the same (1 to 5) proportion and the slices incubated in nitrogen for 90 minutes. The medium is then centrifuged and bio-assayed for vasoactivity. After the 90-minute anaerobic incubation, the slices are exposed to a known amount of active ferritin in oxygen for 120 minutes at 37.5°C. The medium is then centrifuged and bio-assayed as above. Control slices from the same liver are treated similarly except for the omission of the antibiotic.

Figure 18 summarizes the results obtained. In agreement with our earlier work, normal liver slices released vasoactive ferritin into the medium during the anaerobic incubation (9); under similar conditions those briefly exposed to aureomycin did not do so (10). Control liver slices which had been exposed to anaerobiosis, uniformly failed to inactivate added ferritin on subsequent aerobic incubation, while those exposed to aureomycin before anaerobiosis retained their normal

		Blood 0.3 ml	Liver slice incubation: $O_2 \times 60$ minutes	Liver slice incubation: Plus ferritin in $O_2 \times 120$ minutes
VEM	Strong	○○○○○		
VEM	Mod			
VEM	Mild			
	Neutral	●	○○○○○○	○○○○○○
VDM	Mild	●	●	●
VDM	Mod	●	●	●
VDM	Strong	●●●	●●●●	●●●●

○ = Aureomycin ● = Controls

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blood samples from the control group usually contained VDM (vasodepressor material)

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Shorr The livers were excised at the end of the shock experiments sliced, and incubated aerobically in Ringer phosphate. Those from shocked controls released vasoactive ferritin aerobically as if the Pasteur mechanism were abolished whereas livers from the aureomycin-treated rats did not release ferritin under these conditions they behaved like the livers of normal animals.

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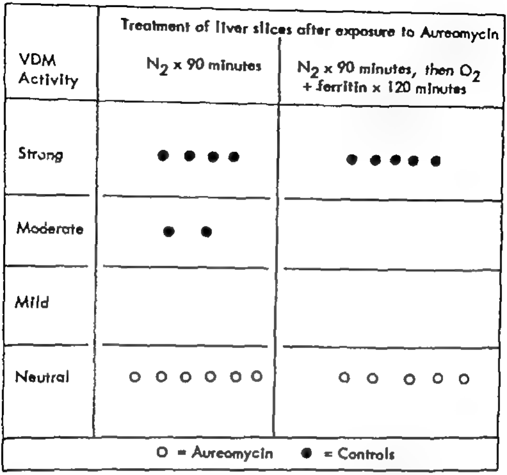

FIGURE 18 *In vitro* effect of aureomycin on the hepatic ferritin systems. Liver slices (1 gm.) incubated for 10 minutes at 37.5 C. with aureomycin (10 μg.) in 5 ml. Ringer phosphate medium in oxygen. Slices washed twice and reincubated.

ferritin inactivating capacity Thus besides its known antibiotic activity aureomycin seems to possess a direct action at a cellular or enzymatic level on the hepatic ferritin formation and inactivation systems

Shorr It is clear that a 10-minute aerobic exposure of the liver slices *in vitro* to these minute amounts of aureomycin (2 μg./ml or 10 μg./gm. liver) prevents the release of vasoactive ferritin upon subsequent anaerobic incubation, something which normally occurs under anaerobic conditions it also prevents the deterioration of the capacity to inactivate ferritin which normally occurs when the liver is incubated for 90 minutes in nitrogen and then put back in oxygen

Levine Dr Shorr your assumption is that there is an oxido-reduction process going on, ferrous $\rightleftharpoons$ ferric, and that exposure to nitrogen inhibits it However despite the anoxic exposure aureomycin gives

the liver back the ability to reoxidize the ferrous form of ferritin What kind of process would be able to accomplish this?

Shorr You mean, do we know how or by what mechanism aureomycin prevents the reduction of ferritin? We can only speculate about that at present

Nickerson The oxidation of the added ferritin occurs in the presence of oxygen.

Shorr The inactivation capacity is tested in oxygen

Fremont Smith They retain the capacity when put back into the oxygen

Levine In the first column of Figure 18 there is no putting back.

Shorr That is anaerobiosis the normal liver releases vasoactive ferritin, the aureomycin treated liver does not.

Nickerson In that experiment reduction is prevented, but oxidation does not occur

Baez There is thus an interesting parallelism between the *in vitro* and *in vivo* protective effects of aureomycin on the ferritin formation and inactivation systems the preservation or prolongation of the compensatory peripheral vascular readjustments during the hemorrhagic experiments, and the survival rates These observations with aureomycin are regarded as supporting the concept advanced by our laboratory that the alteration of the hepatic ferritin mechanisms brings about consequences of major importance for the ability of the animal to survive a standardized shock procedure.

Shorr There is thus at least one other specific action of aureomycin in the organism which takes place at concentrations lower than are ordinarily considered to be antibiotic.

We have studied the ferritin mechanisms because of their deterioration in shock but we do not mean to infer that aureomycin may not affect other systems We have demonstrated that treatment with aureomycin is associated with the preservation of the ferritin systems during *in vitro* or *in vivo* hepatic hypoxia. Administration of these small amounts of aureomycin *in vivo* appears to have only a brief indirect influence on the behavior of the terminal vascular bed in the mesoappendix. Nevertheless its injection prior to shock is associated with the preservation of a compensatory type of peripheral vascular response throughout most of the drastic hypotensive period in contrast to the deterioration of the circulation in the control animals under these circumstances Our inferences are that metabolic products of hepatic hypoxia including ferritin, are prevented from appearing in the aureomycin-treated animals and that in their absence there is preserved a type of peripheral vascular behavior favorable to recovery from shock

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SHOCK IN GERM FREE RATS

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FOR SOME TIME it has been obvious that the nature of the bacterial contribution to the evolution of the shock syndrome could be studied ideally in an animal such as the rat if its background was rigorously controlled with respect to bacterial contaminants This unique situation existed only in germ free colonies of the type maintained at the Lobund Institute of Notre Dame under the direction of Dr James A. Reyniers A preliminary series of hemorrhagic shock studies was therefore instituted in 1953 and continued at intervals through 1954 and 1955 Collaborating with us in the shock studies on the rat were Dr Helmut A. Gordon and Morris Wagner of the Lobund staff Because of the unusually complicated nature of the experimental procedure in such germ-free studies only a comparatively small number of rats (20) have been investigated to date

Before entering into the details of the experiments a number of points should be made clear concerning the germ free rat *per se* As might be anticipated rats reared under germ free conditions differ from conventional animals in a number of important respects These animals have been maintained on a semisynthetic diet which has been rigorously processed to rule out bacterial contamination. As an example of differences in this regard can be cited the unusual size of the cecum of the germ free rat, the diminished connective tissue in particular regions and the failure of the lymphatic system to develop fully in the intestinal tract.† Thus from several points of view the germ free animal represents a new species of rat, as it were, so that a direct comparison in terms of a completely satisfactory control experiment cannot be readily achieved In an attempt to match these animals with comparable conventional controls certain difficulties arose Foremost among these was

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†Gordon, H A. Unpublished data.

the fact that the use of the semisynthetic diet in the conventional stock colony did not produce animals which were comparable to the germ-free ones in those given a semisynthetic diet after adulthood is reached a loss in vigor sets in, together with a tendency toward the development of respiratory infections, but a practical diet (Rockland) that offered excellent results in the stock colony did not appear as desirable in the germ-free colony In the present experiment, the decision was reached to use the best germ free and conventional stock of comparable age, body weight and sex disregarding accordingly strain and diet Such considerations, in general illustrate the difficulties in the selection of conventional animals which can serve as controls to the germ-free It appears that for this purpose the best we can secure is a comparative background offered by a variety of conventional animals which represents different strains and environmental conditions From this background it is possible then to establish the latitude or response which the conventional organism can muster to the variable under investigation it is against this background that the germ free animal can then be gauged. The presently used conventional animals accordingly represent only one point in this spectrum therefore, by themselves, they should not be regarded as valid controls to the germ free animal

In setting up the shock experiments a good deal of improvisation was necessary in order to avoid bacterial contamination during the handling surgery and bleeding procedure This phase of the program was worked out by Dr Trexler of the Lobund staff

Routine in these experiments were bacteriologic samples and gross and microscopic autopsy of the animals In addition to the regular germ free rats rats contaminated with a fastidious pleomorphic bacterial organism were also used This organism could be cultured routinely from fresh cecal contents but only sporadically from freshly voided feces and never from the organs body surfaces or cage environment Since this organism had no apparent effect on the germ free rat characteristics described experiments were also conducted on these so-called monocontaminated rats Their history diet handling etc., were the same as the germ-free animals Anesthesia was induced by intramuscular pentobarbital (3 0 mg /100 gm. body wt) The carotid artery was exposed and cannulated for removal of blood and blood pressure measurement Graded bleedings served to induce a state of hypotension which was maintained at selected levels by the conventional set of calibrated pipets arranged as a self regulatory bleedout infusion system

In the Lobund experiments graded hemorrhage was therefore studied in germ free rats monocontaminated rats and conventional rats under sterile precautions and in conventional rats both on a semisynthetic and

on a stock diet under ordinary laboratory conditions A number of germ-free rats were also removed from their usual environment and kept in the laboratory for varying periods before hemorrhage was induced

In general, the findings clearly indicated that the reaction of the germ free rats to hemorrhage was similar to that of their conventional counterparts Germ-free rats tolerated blood loss neither better nor worse than standard rats when subjected to an episode of drastic hypotension at 35 to 40 mm. Hg for periods of 3 hours The characteristic pattern of decompensatory uptake of blood from the blood reservoir developed after 1 5 to 2 0 hours of drastic hypotension. Following blood replacement and closure of the neck wound, these animals were left in their germ free quarters either to terminus or were sacrificed 24 to 48 hours later A period of 3 to 4 hours at 35 mm Hg was selected since this episode provided in control animals outside the sterile cage a mortality of 65 to 70 per cent Almost identical statistics were obtained in germ-free experiments carried out inside the special plastic hood Careful bacteriologic studies of the cage interior animal tissues and blood specimens failed to indicate any bacterial contamination.

Frank That is in neither group?

Zweifach Conventional rats subjected to shock under ordinary laboratory conditions were found to have a number of different bacterial contaminants in either the blood, liver or spleen. When conventional animals were subjected to hemorrhage under rigidly aseptic conditions no bacteremia developed in blood liver or spleen

Monocontaminated rats, bled under aseptic precautions in the sterile hood, showed a similar reaction to the shock procedure as did germ-free rats We could not on the basis of either survival studies or the tolerance to blood loss during protracted periods of hypotension distinguish between monocontaminated and germ free rats in these experiments.

Autopsy findings revealed the typical congested bowel It should be pointed out that germ free rats differ from conventional rats in having an unusually large cecum Apparently in the absence of bacteria the cecal wall thins out and becomes distended This organ following hemorrhagic shock, usually showed evidence of considerable congestion and of hemorrhagic material in the bowel lumen

Another difference was the finding that the liver in many of the germ-free rats at death was comparatively pale and contracted as compared with the dark congested and enlarged liver in conventional

controls Several of the germ-free rats showed a moderate amount of liver congestion following blood replacement and circulatory collapse. Germ free animals which survived the procedure had little or no bowel pathology

Fine Was there a difference in the amount of blood in the cecum of the germ free rats as compared to that in the contaminated rats?

Zweifach In some of the rats definitely so In others, the cecum was free of hemorrhage

Fine How great was the difference?

Zweifach I would say that a significant proportion of the intestinal hemorrhage in germ free rats was in the cecum, whereas in the normal rat the hemorrhage and congestion usually was located in the small intestines.

Fine Do you know whether the germ-free rat lost enough blood into his cecum to account for his subsequent collapse?

Zweifach Although we had no measurements of the amount of blood present in the cecum, my impression was that the loss here was not sufficient to account for the irreversibility

Fine This factor may still be responsible for the failure of blood replacement to restore the circulating blood volume

Zweifach The amount of cecal hemorrhage was a variable factor Furthermore sufficient numbers of germ free rats were encountered with little or no cecal hemorrhage despite irreversibility to rule out this factor as an unusual feature of the syndrome in these animals Although this condition may represent a contributing factor which altered the picture somewhat in particular animals it definitely cannot be considered a major factor in this respect

Baez. May I ask whether the blood was in the lumen or the wall of the cecum?

Zweifach There may be minor amounts of free blood in the lumen of the cecum

The wall of the cecum in the germ-free rat is unusually thin Hence although it shows signs of congestion and stasis the amount of blood contained therein cannot be very large

Fine You do not know what the circulating blood volume was at the time of death?

Zweifach As far as we could tell the blood pressure returned to levels of from 100 to 120 mm Hg and remained in this range for at least 1 hour following blood replacement We have no direct information on the amount of blood in circulation thereafter

Fine What I should like to know is whether it is possible that subsequent bleeding into the gut wall or into the lumen of the gut *per se*

reduced the volume of blood in circulation sufficiently to account for the collapse of the germ free rat

Zweifach In all, we have conducted a series of about 20 animals under germ free conditions Only about a third of these animals showed this unusual hemorrhage into the cecum. The remaining two-thirds did not show sufficiently extensive bleeding or congestion into the cecum to support your assumption

Green Did you bleed both groups of animals down to constant level of pressure and then reinfuse them when they had taken up a certain amount? Did both groups of animals require the same amount of time before they took up 50 per cent?

Zweifach These experiments were carried out on a time basis, the rats being maintained for a period of 3 hours at drastic levels of hypotension During this period they took up variable amounts of blood from the reservoir Occasionally when their uptake was extensive, the rats succumbed before the end of the experiment In the majority of instances, the experiments were arbitrarily terminated by blood replacement after 3 hours of drastic hypotension, a procedure which, on the basis of our previous experience, results in about a 70 per cent mortality

Green Did the germ free and conventional rats take up similar amounts of blood during the 3-hour period?

Zweifach We could not establish any statistically significant differences either in the uptake tendency or in the extent of blood loss tolerated

Rappaport Was there a difference in the vasomotor behavior between the germ free and the conventional rat?

Zweifach Microscopic visualization of the circulation in the mesentery was carried out in only two instances in order not to complicate the survival statistics We therefore have no meaningful evidence along these lines

Shorr Did you treat any of the germ free rats with aureomycin?

Zweifach No

Engel In view of the liver changes which you described did you look for evidence of metabolic alterations in the livers of germ-free animals? For example, do they show a rise in plasma amino acids as do conventional rats?

Zweifach *In vitro* studies on liver metabolism have not as yet been carried out. Blood samples were not taken either for bio-assay or chemical determinations

Engel Plasma amino acid levels should represent a simple basis of comparison between the two groups of rats

Baez. Since these animals are germ free, I wondered whether their lymphoid tissue might be underdeveloped and whether this might account for the decrease in the thickness of the wall and the over all distention of the cecum.

Zweifach According to Dr Gordon, the thinness appears to be attributable to a relative paucity of connective tissue and to a marked loss of tone of the smooth muscle elements

I should like now to discuss several other experiments carried out with respect to the bacterial elements in hemorrhagic shock These were conducted *in collaboration with Dr S G Hershey Dr W Antopol* and Dr I Saphra of Beth Israel Hospital Rats subjected to hemorrhage under standard conditions were found to show a variable bacteremia in which only bacteria of the type present in the bowel appeared in the blood liver and spleen irrespective of a reversible or irreversible out come A series of extensive elimination experiments demonstrated that the contamination was the result of organisms introduced exogenously through the bleedout reservoir and the arterial cannula. It was found that in the process of handling the rats, the experimenter contaminated the cannula and the blood pressure attachments This resulted in a systemic bacteremia in a fair proportion of routine shock experiments

In order to avoid contamination of this sort, it was necessary to utilize rigid asepsis When every piece of apparatus was disassembled and autoclaved and conventional sterile surgery used no bacteria could be detected in either the tissue or blood specimens obtained during shock and as late as 48 hours following blood replacement Despite such precautions and the elimination of a bacterial element an irreversible type of hemorrhagic shock could be induced regularly in these rats

Fremont Smith Is it more difficult to produce irreversibility under aseptic conditions?

Zweifach On the basis of our previous experience, hypotension at 40 mm Hg for from 2 to 3 hours uniformly resulted in a lethal out come to 70 per cent of our rats When the identical procedure was carried out under aseptic precautions, 70 to 80 per cent of the animals now recovered following blood replacement However when the hypotensive episode was extended for an additional hour the syndrome became highly lethal again without intervention of bacteremia

Fremont Smith Do these animals now die acutely or do they die several days later?

Zweifach The rats usually succumb during the night or on the following day *i e* from 4 to 18 hours later Another set of experiments

also bears on the relative importance of the bacterial element in shock. As previously mentioned, the rat subjected to shock under conventional circumstances shows a significant incidence of bacterial contamination (*E. coli, Klebsiella, enterococci,* and occasionally *paracolon*). These rats can be protected against the lethal outcome of a standard hemorrhage by a number of modalities including pretreatment with dibenzyline or chlorpromazine (1), which agents served to counteract the irreversible trend of the syndrome despite the fact that cultures of the blood, liver and spleen continued to show the same type of bacterial contamination present in unprotected rats. The data suggest a secondary contributary role of bacteremia in the development of the syndrome.

The particular bacterial contaminant which appears in the blood and tissues during shock was found to vary considerably. For example, the bacterial contaminants that entered into the picture in rats studied at Notre Dame were different from those encountered in our New York animals. Rats in a third laboratory have shown a still different pattern of bacterial involvement. This factor may depend on the conditions under which the rats are kept, the diet and even on the particular strain.

A number of different conditions serve to predispose rats to hemorrhagic shock, including agencies such as cortisone, sublethal roentgen radiation (350 to 400 r) or adrenalectomy. When such animals were subjected to hemorrhagic shock, bacterial elements appeared regularly in the blood, liver and spleen, despite rigid aseptic precautions. For example, following exposure to roentgen rays (10 to 30 days after 400 r) the bloodstream and tissues of rats subjected to hemorrhagic shock showed contamination with organisms from the respiratory tract such as *Pseudomonas aeruginosa*. In adrenalectomized rats bacterial elements from the intestinal tract appeared in the liver, spleen and blood following an episode of hemorrhagic shock.

Shorr: Would that hold for the liver too?

Zweifach: Bacteremia could be demonstrated most regularly in the liver and spleen, and less consistently in the blood stream.

Nickerson: The rats subjected to hemorrhage in New York under sterile conditions seem to survive better than the completely germ free rats.

Zweifach: The first series of experiments at Notre Dame were carried out at an extremely drastic level of hypotension, 35 mm. Hg for 3 hours. Under these conditions there were no differences in survival statistics between the germ free rats and our own animals at New York University. Subsequent experiments with the germ-free rats were con-

ducted at blood pressure levels of 40 mm Hg for 3 hours These rats show comparable survival statistics when compared with conventional rats at Notre Dame and with our stock rats at New York University A direct comparison between Notre Dame experiments and our own data is not possible since these were done on different strains of rats (Lobund and Holzman strain at Notre Dame, and Wistar at New York University)

Nickerson I should like to comment on what may have been a misconception relating to dibenzyline treated animals in Dr Horvath s original question If the animals are properly pretreated the amount of bleeding may be identical with that of the controls Although the survival rate is increased, they do not necessarily bleed out less

Engel What was the per cent mortality in the germ free animals?

Zweifach In experiments conducted at 35 mm Hg for 3 hours 70 to 75 per cent mortality was obtained, a figure identical for conventional rats studied under the same sterile precautions When the germ-free animals are subjected to a hypotensive episode for 3 hours at 40 mm, Hg about 50 per cent survived following blood replacement a figure comparable with control experiments

Engel If the shock were made less severe, would there be a higher mortality in conventional animals as compared with the germ-free ones? It is obvious that if an animal is bled too drastically the mortality will be high under any circumstances

Zweifach Our initial experiments were conducted on the assumption that the germ free rats might tolerate shock better than the conventional animals. For this reason 3 hours at 35 mm Hg was used as a test procedure. This level of hypotension is not so drastic as to be uniformly lethal under any circumstance For instance we have found that rats pretreated with chlorpromazine tolerated this degree of hypotension and showed a high percentage of survival

Several sets of experiments were carried out in which we attempted to make stock rats resistant to hemorrhagic shock by pretreatment with endotoxin These animals were given successively larger doses of endotoxin (meningococcal or *E coli*) at 48-hour intervals until they had received five doses of the material The final dose was a relatively large amount (1 mg) These animals were subjected to hemorrhagic shock 48 hours later No protection could be demonstrated by this type of pretreatment It should of course be emphasized that the resistance to endotoxin may be both dose and time-dependent Further studies along these lines are indicated

Frank Dr Zweifach I can support your statement about the absence of bacteria in shock experiments carried out under aseptic precautions

Our largest experience in that regard was with dogs When we took repeated samples of arterial blood, vena cava blood at the level of the hepatic veins portal vein blood, and thoracic duct lymph all through hemorrhagic shock experiments lasting 5 6, or 8 hours, we found consistently negative cultures In a few experiments we took con tinuous samples throughout the shock period and these remained nega tive In a search for just a single organism or very few we interrupted several experiments by exsanguination at different stages of shock with culture of samples over 100 ml in volume, but were not able to find bacteria in the blood or lymph while dogs were alive, although bacteria were found in portal blood and less regularly in systemic blood very soon after death.

Zweifach We have some preliminary evidence regarding the ability of drum-resistant rats to tolerate endotoxin These animals were made resistant by successive exposure to increasing doses of drum trauma over a period of 10 to 12 days until they withstood 1000 drummings a dose lethal to normal rats Two types of experiments were conducted. In one 5 7 5 8 and 10 mg of *E coli* endotoxin doses which were LD_{100} in control rats were administered intraperitoneally to several groups of drum-resistant rats No significant resistance to the lethal effects of this range of endotoxin was demonstrated In a second set of studies drum-resistant rats were given varying doses of endotoxin 1 to 3 hours prior to drum trauma We were unable to demonstrate any undermining influence of pretreatment with 1 to 2 mg *E coli* extract on the resistance of these rats to drum trauma. These findings are in contrast with comparable experiments on normal rats where 1 to 2 mg of endotoxin shifted the mortality figures from 30 per cent in un treated controls to 90 per cent in treated animals

Frank Did the drum resistant rats tolerate hemorrhagic shock better?

Zweifach We have not carried out such experiments

Shorr Yes we found that rats made resistant to drum trauma were also more resistant to hemorrhagic shock (2)

Fremont Smith The rats are more resistant and also they show the same liver changes?

Shorr Although at the end of the hemorrhagic experiment both the resistant and the control rats had VDM in their blood the livers of the resistant rats retained the ability to inactivate added ferritin in con trast to the controls which had lost this inactivation capacity Livers of resistant rats (not subjected to hemorrhagic shock) retain their capacity to inactivate added ferritin after drumming or following a 90- or 120-minute exposure to anaerobiosis *in vitro* whereas normal liver loses this capacity under similar conditions

Horvath As I understand this germ free rats and conventional rats from the Notre Dame colony showed no differences in their ability to tolerate shock. Rats studied at New York University under aseptic conditions however withstood hemorrhagic shock better than those examined under standard conditions Am I right in this?

Zweifach I would rather not compare the over all statistics with respect to tolerance to shock in experiments conducted at New York University and at Notre Dame. Each of these sets of experiments requires its own specific controls

Horvath Would you say that the two sets of data are in conflict with respect to the importance of the bacterial factor?

Zweifach No The experiments at Notre Dame were carried out under comparable conditions of sterility This made it possible to compare rats reared under germ free conditions with conventional rats In our own laboratory rats of the Wistar strain were found to tolerate hemorrhage better when bacterial contamination was avoided In this instance the predisposing element was an exogenous bacteremia introduced by the experimental procedure When this factor was eliminated, a standard form of irreversible shock could then be set up without the elements of bacterial contamination entering into consideration

Green Dr Zweifach, I should like to ask you to compare the degree of exposure to hypotension in the germ free rats done conventionally but with sterile or aseptic conditions outside the asepsis cage against the rats in your laboratory which were handled in the same way Did the presence of bacteria in your animals make them any more or less susceptible to induction of the state of shock?

Zweifach In my estimate the Holzman and Lobund strain of rats at Notre Dame were somewhat more susceptible to shock of this type than our own Wistar rats

Horvath By that you mean the volume of blood removed from the Notre Dame animals was less?

Zweifach I think that the chief difference between the Wistar and Holzman rats falls in the category of the tolerance to a period of hypotension before uptake from the bleedout reservoir develops The decompensatory uptake of blood was definitely greater in the Notre Dame experiments than in our own rats subjected to hemorrhage under aseptic precautions

Nickerson Strain differences are extremely important in drum shock experiments For example in rats which we have used this year the lethal dose (L.D $_{50}$) was 400 to 450 revolutions, in contrast to previous years where the L.D $_{50}$ dose was 700 with another strain of rats

Burton In listening to this obvious conflict of point of view between Dr Fine and others as to the importance of bacterial factors it strikes me as most unfortunate for the settling of this conflict that the aspect of cost enters that it is necessary to use the rat, because I think Dr Fine would say that the rat possesses a natural resistance to bacterial infection, and other animals do not the rat s properdin levels are much higher So while we may prove that in the rat the bacterial factor is not a very important thing it still does not really tell us the importance of the bacterial factor in other animals does it?

Zweifach There is a good deal of evidence to support the contention that the hemorrhagic shock syndrome develops along comparable lines in the rat and the dog Actually the dog may be an exception rather than the other way round because of the enormous number of bacterial contaminants harbored by this species

Shorr Nevertheless the rats die

Haist I should like to ask Dr Burton what he thinks these rats died of if they didn t die from shock.

Shorr Are you going to ask Dr Burton to give a theory of shock?

Burton. I am a neutral I think that even if all the bacterial factors that are most important in some animals are removed, the animals obviously would still die, wouldn t they?

Haist They do not die immediately they die after a period of time

Shorr And the process can be reversed or prevented by a variety of agents

Burton We discussed this at the first conference and we all agreed eventually that there were limiting factors, and that it might be found that in certain circumstances and certain kinds of shock in certain animals one vector was the important factor Even if that factor were removed the animals would die of one of the other factors It might take more bleeding to do so Was this not the feeling we reached in the first discussion?

Shorr I think this was again a matter of definition and of our recognition that our terms are of necessity limited by the specific conditions under which we operate at a particular time

Srikantia It might be relevant to make an observation here on the experiments by Dr Fine and his group using the inactivated aureomycin. I think they inactivated the antibiotic by keeping saline solutions of the drug exposed to the atmosphere for several days at 37 C. The antibiotic property of this material fell to about 1/12 500 of the original aureomycin. Using this inactivated aureomycin for hemorrhagic shock experiments they found that the degree of protection afforded by it was almost as high as the degree of protection

obtained by the "unaltered aureomycin This experiment would tend to shift the emphasis, if any from the bacterial factor as a major contributory cause for the development of irreversible shock

Frank No The experiments with "inactivated aureomycin did seem to some of us at least to indicate a protection, but by no means the kind of protection that the unaltered aureomycin conferred

The material which we called inactivated aureomycin was something that had been incubated in solution for a number of days. We do not know how its molecular structure was altered and what relationship to aureomycin it still bore It did seem to us, after studying the data and applying the usual statistical tests that there was some protection As I say it was nothing like the protection of the original material

The question was what was the significance of this? It seemed open to at least two interpretations One was that the material was working by a nonantibacterial mechanism The other was that the *in vitro* test, which shows that a given material is not antibacterial is not in fact an accurate index of what that material may do as an antibiotic in an animal s body We tested the material both before we gave it to the dogs and also in plasma samples because of the possibility that feeding or injecting it might reconvert it to an active form We found no evidence of antibacterial activity in either test But still it can be argued that so great is the sensitivity of the shocked animal that even a minute residual antibacterial action unmeasurable *in vitro* may still be protective I think the matter lies in this unresolved position as far as this experiment alone is concerned The alternative difficulty is to explain how several antibiotics of widely varied chemical structure protect in shock by a common mechanism other than their

time that the rate of destruction of bacteria becomes less than the rate of production of bacteria by multiplication, the total numbers of bacteria begin to rise (3)

Horvath Isn t it also true that the rate at which the bacteria get to the liver from the gut is also going to be reduced?

Knisely That is what I tried to say

Horvath Also the rate of destruction may be different

Knisely One limitation on the rate of phagocytosis is the concen tration of organisms per milliliter of the blood which enters the liver multiplied by the number of milliliters of blood passing into the liver

Dobson In regard to the ability of the liver to clean the blood (that is free it of bacteria) some insight may perhaps be gained from consideration of colloid studies which we have carried out at Donner Laboratory (4,5 6) Using radioactive colloidal chromic phosphate, Dr Warner and I find that, in the normal dog, the half-time of removal of colloid from the blood stream is one and one half minutes If a dog is bled this disappearance rate remains essentially the same The liver blood flow has indeed decreased but the decrease is approximately proportional to the extent of the bleeding Thus the disappearance rate constant which is the fraction of the blood volume perfusing the liver per unit time, can remain the same even in the presence of a reduced liver blood flow For this reason, the blood may be just as rapidly cleared of colloid or bacteria after hemorrhage as before.

On the other hand a reduced blood supply might interfere with the ability of the liver cells to maintain their normal function Thus liver blood flow can be considered from two different aspects one of supply ing needed metabolites to the liver cells and one of maintaining a close equilibrium between this important metabolic organ and the other body tissues (6) If the reduced blood flow occasioned by hemorrhage were to interfere with the metabolism of the Kupffer cells and thus reduce their ability to phagocytize, then the liver would be expected to be less efficient in clearing the blood of colloid But since the phagocytic cells have been shown to function efficiently at a greatly reduced blood flow the liver is able to clear colloidal chromic phosphate from the blood rapidly

Shorr At a given time or the same amount of colloid?

Dobson The colloid disappears at a constant rate over a period of about 6 minutes, which is sufficient time to reduce the concentration in the blood to about 5 per cent of the initial value In regard to the amount of colloid we find that the rate of removal is independent of the quantity of material present until very large quantities are used (650 μg in a mouse) (5)

period of irreversibility? Does the half time for removal of colloid still remain 1½ minutes, Dr Dobson?

Dobson We really have not studied hemorrhagic shock We were trying to determine whether blood loss could account for the observed depressions of cardiac output and liver blood flow in our burned animals and we decided that it could not (8) We have made no attempts to measure reversibility or irreversibility But since we generally removed only about 30 per cent of the blood it is safe to say that we certainly operated in the reversible period Probably in most cases the animals were not even in shock I use this term with some hesitation Blood pressures were seldom measured, but when they were, they were not down appreciably Cardiac output, however, usually was markedly depressed, though not as much as in the burned animals with a comparable blood loss

Shorr Of course, one of the objects of inviting you here, Dr Dobson was to persuade you to work in the field of hemorrhagic and traumatic shock!

Zweifach One cannot overlook the fact that the shock syndromes in the rat and dog have a great many features in common The same modalities or contingencies that protect rats against irreversible shock also protect the dog despite differences in their bacterial flora Both the rat and the dog can be protected against lethal hemorrhagic shock by pretreatment with chlorpromazine or dibenzyline, irrespective of the presence or absence of bacterial infection *

Burton My reply to that is that the death from shock is caused by a combination of many adverse factors The protection by a given agent may be because it deals with one of these contributing factors

Zweifach The complexity of the shock reaction makes it essential to separate contributory or sustaining factors from the basic elements of the syndrome This unfortunately has not been possible to date.

Burton Nor should one, by the same philosophy overemphasize the V D.M (vasodepressor material)

Fremont Smith The important thing is not the question of over emphasis or underemphasis but of specificity What we need to answer is where does the bacterial situation enter and how much and where does the V D.M enter and how much? There are many factors to take into consideration including bacteria and V D.M

EDITOR'S NOTE The following remarks are an expansion of the comments given at the conference

Knisely Most of the logic which today is being used in the study of

*Zweifach, B. W., and Hershey S G., Unpublished data.

shock is not relevant to the problems presented by circulatory shock. We are hunting for the direct causes of shock or of circulatory failure Our ultimate purpose is to try to find out how to prevent circulatory shock or circulatory failure In order to straighten out our thinking on these points, I should like to talk about two different classes of causation Let us call one "positive causation and the other negative causation." Most of the logic commonly in use in our civilization seems to be a part of positive causation. Something positive is done which, through a chain of positive effects, ultimately directly causes one or more end results.

As an example of positive causation: A man exerts force on the accelerator pedal of his car The pedal, through a system of levers, opens a valve which admits more air and gasoline to the combustion chambers of the engine. The engine converts this fuel into energy and delivers it to the back wheels, and the automobile goes faster Every step in the above chain of events is a part of positive causation.

As an example of negative causation, a young man arrives in a great city with $300 in cash in his pocket. Some of this money goes for food and wine and some for entertainment. A little money is lost here, a little there, and 2 days later there is no money left.

In dealing with problems of positive causation, one may ask, "What factors are *necessary* and *sufficient* to produce a given result?" And one does not understand the steps in the causation until he understands every factor which is *necessary* and which together are *sufficient* to cause the final end result.

When dealing with negative causation, any single factor may be sufficient, and any combination of fractional losses may add up to sufficiency and, strangely enough, no one of the factors which may be present need be at all *necessary to cause the result. Our young man* could have spent all of his money on wine, or all of it on song, in order to arrive at financial insolvency

An animal or a man receives a series of pounding blows to the legs or body or a series of severe burns over the outside of the body and after a time his circulation fails" (9 10) We have a tendency to say "What *caused* the circulatory failure? One group of investigators points out that there was a sharp decrease of blood flow through the liver Another points out that the blood is agglutinated making it difficult to pump through small vessels. Other groups point out that there is a "pooling" of blood in the abdominal cavity Other groups of investigators dealing with other experiments find that one or more of the factors designated above is completely absent in still other cases of complete circulatory failure

My own thinking about circulatory failure has taken a new and, I believe profitable turn by examining each and every kind of situation in which the circulation is known to fail, in terms of negative causation. Each individually known pathologic circulatory factor causes how much of a circulatory decrement in this individual animal or man? How many different kinds of pathologic events can be listed, *each of which* can cause a circulatory decrement? How do these decrements summate? I shall list only a few Spasms of pulmonary arteries can decrease the

flow of blood into pulmonary veins and the left heart, thereby decreasing cardiac output (11) The plugging of great numbers of small pulmonary artery tips with masses of agglutinated blood cells also can decrease the numbers of pathways between the right heart and the left heart (12) The contraction of hepatic outlet valves, whether sinusoid outlet valves, Deysach s small sluice channels, or big hepatic veins can sharply decrease the flow of blood into the inferior vena cava. This would tend to fill the liver and dam blood within the portal vein bed. The plugging of great numbers of tips of the portal vein within the liver by means of masses of agglutinated blood cells has been observed and forcibly reduces the amount of blood passing into and through the liver and through the hepatic veins into the inferior vena cava (3 13)

Hemorrhages cause a loss of blood so that there is less blood to pump The leaking of whole blood or of the fluid parts of the blood into crushed or burned tissues also decreases blood volume.

The settling of enormous amounts of agglutinated blood cell masses to the lower sides of vessels where they become stationary decreases the numbers of red cells which are circulating and carrying oxygen (14 15)

Hypoxic small blood vessel walls leak the fluid parts of the blood (16-23) and this decreases circulating blood volume"

The hemoconcentration of the blood, whether unagglutinated or agglutinated, necessarily increases the resistance which this blood offers to being pushed down through long narrow cone-shaped arteries and arterioles. This is not an increase in "viscosity or of apparant viscosity" but it is a change in the physical consistency of the blood of such a nature that the blood resists passage through vessels. The fact that increasing red cell concentration causes increased resistance to being pushed forward was first published by Whittaker and Winton (24) and recently by Jeffords and Knisely (25) *

Spasms of arteries in many parts of the body either partial or complete, contribute to a stoppage of blood flow or a reduction of forward flow and thereby decrease the rate of supply of blood to the linings of vessels and initiate hypoxic states of small vessels and still further leakages.

Toxins, for instance rattle snake venom, can cause small blood vessel linings to leak (this is positive causation) the leakage results in a decrease in circulating blood volume (negative causation)

It is not my purpose here to make a total list of possible factors which can occur in different kinds of shock. For another list, see Knisely *et al* (26) I should like to point out, however that when dealing with negative causation, any single factor when operating maximally can, by itself cause the circulatory failure For example, members of my laboratory have bled animals and men (27 28) keeping trauma at a minimum (only a needle stick) and they found that following hemorrhage alone there is no agglutination of the blood cells Every

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* The investigations in support of studies of the pathologic circulatory physiology following severe burns are supported in our laboratory by Navy Grant Nonr-434(01) and United States Public Health Service Grant H 1683(C)

one of us is capable of believing that complete circulatory failure can be caused by hemorrhage alone In some kinds of circulatory failure groupings of the above-listed factors operate, each contributing its own share to the decrease in venous return to the heart cardiac filling, and cardiac output.

In many situations, we are dealing with the summations of decrements. Our real problems are Which particular decrements are operating in any one situation? Which summation of the decrements present in this case are together sufficient to cause the total circulatory failure?

It is possible to have complete circulatory failure in the total absence of one or more factors which each of us has believed for a long time to be a part of the shock picture.

In dealing with *negative causation and summations of decrements, no single factor is "necessary* in order to cause the circulatory failure.

The above makes it possible and necessary to determine exactly what each of the specific bacterial toxins which may be present actually does in the injured individual Perhaps, for instance one set of bacterial toxins can damage the linings of small vessels in such a way that they will begin to leak and to sufficient degree so that enough fluid loss will occur to give an inadequate venous return.

Dr Fine s observations are most important We may not choose to agree with him on some point or other but we cannot neglect the bacterial factor in shock in war time Logic here must be aimed toward thinking clearly about summations of decrements some of them large in one kind of shock, some of them small in other kinds of shock.

Shorr I agree I do not think we have any right to neglect any fac that is consistently observed to influence the shock syndrome.

Knisely Or if we discover a factor which can decrease venous ret first in a rat then we have the *privilege* and *obligation* of looking the presence of that factor and measuring its magnitude in each of kinds of shock in man

Burch Some of the human syndromes are different Some of shock like states encountered in man are different from certain ty of experimental shock

Fremont Smith I think Dr Zweifach s point that when an ag protects in rat and dog and protects with or without infection should therefore be very much concerned with finding out how mu further we can go with that agent and how much it will prot against infection also

Knisely I agree with that

Hurst Isn t there some slight difference in viewpoint here? I thi some are concerned with those conditions which are *essential* for t development of irreversibility and death and others are concerned w those factors which *contribute* to the development of shock The may be many factors contributing to the development of shock b

surely ultimately there will be some change which leads to irreversibility and death That is what we should be looking for That is I presume what we *are* looking for and what the argument was about I do not think we should consider that all the factors which will contribute to the development of shock are necessarily fundamental to the development of irreversibility

Shorr Or are of the same importance

Fremont Smith I should like to say this concerning irreversibility we talk as though there were definitely an irreversibility I think there is no such thing There is irreversibility with respect to what? We have already moved from finding what was previously irreversible is no longer irreversible if something else is done So the term in itself is a very bad term. The state is no longer irreversible if aureomycin is given.

Haist Let us say that ultimately all of these animals will reach a stage where treatment will not promote survival They die.

Fremont Smith After they are dead you can say that.

Haist Then perhaps death is the criterion we should use.

Fremont Smith Which is also hard to define

Haist There may be different procedures which will prolong or shorten the period over which the condition can be reversed let us say by fluid infusion or restoration of blood volume

Fremont Smith Irreversibility of restoration of blood volume As long as you say it that way it is acceptable.

Haist Let us say it is an irreversibility in the sense that it cannot be reversed by any known therapeutic procedure That is all that is meant by irreversibility

Fremont Smith Until aureomycin comes along

Haist It does not do any good whatever in the tourniquet rats

Kinsely May I emphasize one more point about the utilization of the concepts of positive and negative causation In hunting for factors of positive causation we say What factors are *necessary* and *sufficient* to yield a certain result? That is the pattern of logic upon which most of our civilization operates The moment we begin dealing with negative causation we are dealing with summations of decrements Whenever the summation of a series of decrements becomes sufficient to cause failure that constitutes sufficiency At that point no other single factor of any magnitude is necessary One single factor acting maximally may be completely sufficient In which case all other factors are unnecessary That is the point you are making here.

Fine If Dr Zweifach s data are taken to mean that hemorrhagic shock of 3 hours duration in the rat is irreversible for reasons that

have nothing to do with bacteria, then our data and Dr Shorr s on the protective effect of antibiotics will need to be explained on basis of a nonantibiotic action of the antibiotics.

Dr Shorr s view is that this is the case that is that aureom protects by virtue of its effect on the ferritin mechanism in the l I have two reservations to make on the validity of this hypothesis I if Dr Shorr is right, then he should be able to show that the prote effect of penicillin and neomycin can be explained in the same Second since aureomycin does not protect the ferritin mechanism in liver of the rat in drum shock why does it increase the survival ra

Until these questions are resolved in favor of the view that antibiotics act by virtue of nonantibiotic properties I prefer to ad to the view that the antibiotics protect against hemorrhagic shock drum shock by virtue of their antibiotic property If that is so the q tion arises whether the alleged germ free state of the Notre Dame must be accepted as fact. We have already observed that the numbe bacteria or the amount of toxin required to kill an animal wl defenses are down because of shock can be very small indeed The d culty of identifying a few bacteria in tissues is well known I told that to get a positive culture from tissues by the conventio techniques an inoculum of as many as 1000 bacteria is required Un the whole germ free rat were thoroughly mashed up and a sufficie large aliquot taken for culture, I would not be sure that the ani was in fact germ-free Moreover as our liver mash experiments s gest even this might not suffice because artificial culture media are nearly as efficient as animal tissue for facilitation of growth of bact when these are few in number and fastidious in their growth requ ments

Finally evidence that irreversibility to transfusion shock was ind the cause of death in the germ free rats requires a demonstration t these rats were normovolemic or nearly so at death This cannot assumed to be the case until the quantitative analysis of the total blo loss into the wall and lumen of the entire gastrointestinal tract can shown to be volumetrically insignificant

I should also like to discuss the discrepancy between the reports Dr Hardy and Dr DeBakey (29) and our own on aureomycin

Shorr We do not understand what the discrepancy is

Fine I am referring to their report on repeating our experime on the effect of aureomycin in hemorrhagic shock After a considera

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Dr Baez comments after the meeting Please note that there a misunderstanding Dr Fine s part when he says that a reomyc n does not affect the ferr t n s em studies presented by Dr B ez and Dr Shorr at th s meeting show the opposite

number of experiments with variations of which we would not approve, they did their last group of experiments as we would have them done They reported some benefit from aureomycin but it was not nearly as striking as our own They stated that in about 35 per cent of the last series of animals there was a prolongation of survival time, but no survivals (29) This was not a total contradiction of our results but it was very weak confirmation of our own data Although we had done a very much larger series of dogs and had obtained good results with other antibiotics as well, the discrepancy had to be explained

In looking over their data I noticed that about 40 per cent of their dogs showed a bacteremia after the transfusion there were no *Clostridia* but there were other intestinal flora.

This bacteremia was something we never could demonstrate We could find occasional *Clostridia* but no other intestinal flora in the blood during life This indicated that perhaps the flora in their dogs were more resistant to aureomycin than the flora in our own laboratory At any rate, I entertained the suspicion that they were having a very much more difficult time controlling bacteria than we had had Nevertheless it was incumbent upon us to repeat the experiments which we had concluded some 3 years previously Since we had done a great many more experiments than they had and had used all sorts of antibiotics over a long period of time, it was quite possible that we, too had set up a very resistant flora. Indeed we had evidence of it over 2 years ago from our inability to affect the fecal flora with antibiotics When we repeated the experiments in the customary way we could do no better than Dr DeBakey and Dr Hardy

In the meantime, Dr Paul György in Philadelphia published some interesting studies on the effect of antibiotics on the development of cirrhosis or necrosis in the livers of rats maintained on a choline free diet (30) He reported that aureomycin for example, delayed the onset of the liver injury by 128 days He found that many other antibiotics did the same thing it was most interesting that the spectrum of effectiveness of the various antibiotics against liver injury was precisely the same as the one we had obtained against irreversibility to transfusion for hemorrhagic shock in dogs Those that failed in cirrhosis of the liver also failed in hemorrhagic shock

He reported further that as the years went by and the experiments multiplied he found he was getting less and less effect with aureomycin and other antibiotics until about 4 years later when he was getting no effect whatsoever from aureomycin This fits with striking precision our own experience after having repeated our experiments as a check on the data in Dr Hardy and Dr DeBakey s report.

He (Dr György) then took the obvious if trying, way out. He repeated his experiments in two laboratories where this kind of work had never been done before and reproduced his original results

It was, therefore desirable for us to repeat our experiments elsewhere to see if we could get our original results But this was too complicated an undertaking We used an alternative, i e we prefed our animals in the dog kennels from which they were to come Immediately or soon after their arrival and before they could acquire our laboratory flora we did a small series of animals with penicillin and neomycin, and we got our original results

Why did the dogs in Houston show bacteremia while ours did not? As I pondered this along came Dr Pillemer s discovery of properdin I asked him if he would assay our dogs for properdin, which he did We sent him the first six dogs sera coded and five out of the six assayed normally i e., from 12 to 18 units The sixth did not, and it was the only dog that collapsed soon after inducing shock it was dead in 3 hours in spite of transfusion It looked like a normal dog like the rest of them

At the same time we learned that most of the dogs at Western Reserve did not have a normal amount of properdin It looked as if this might be caused by a poorer state of health than in our dogs What was the situation with respect to properdin in the dogs in Houston or for that matter with dogs in various other cities It was obviously necessary to do a geographical study of this question since properdin provides the animal with a considerable amount of natural defense

So Dr Pillemer did a geographical study of the properdin titers of dogs in various parts of the country I should like him to make a comment with regard to this, simply as a conclusion for my remarks

Pillemer The National Research Council asked us to do these experiments They took place in Houston Texas Cleveland and Cincinnati Ohio Boston Massachusetts and Rochester New York The only group of dogs that was under the care of a veterinarian Dr S Michaelson was the group in Rochester and they all had normal properdin titers

I shall give the results 12 to 16 properdin units per ml is considered a normal properdin titer I think only one of your dogs Dr DeBakey out of 12 had normal properdin titers All the others had under one unit of properdin Only 9 Cleveland dogs out of 88 had normal titers 3 out of 24 dog serums obtained from Dr W A Altemeier Cincinnati had normal titers and about 60 per cent of the serums obtained from Dr Fine s laboratory had normal titers

Fremont Smith How did you decide that 12 to 16 is normal?

Pillemer We decided that from the results obtained with the Rochester dogs They are healthy dogs that have been dewormed, given various types of vaccines and kept for at least 8 weeks before being put on an experiment. It is interesting that the nine Cleveland dogs that had high titers were able to survive certain types of surgical procedures that the other dogs were unable to stand

Burch I should like to make a statement parenthetically at this moment about a problem that is important in physiologic research Frequently comments are made about doing research on man If a man is said to be normal, he has been studied very well and the clinical criteria are fairly dependable When we work with so-called normal dogs sent to us from the average animal house, we find relatively few of them are normal This is an important problem in research especially when data from different laboratories are under comparison

It is my opinion that investigators doing clinical research probably have much better control and better known animals than investigators in general working with dogs obtained from our present sources and employed in the present manner This is not true for all investigators but appears to be true for most of them

In the case of rats the situation is quite different. Good strains of healthy rats are available in most laboratories A mongrel dog collected from the streets is not carefully examined (blood count, urinalysis, stool serologic studies roentgenogram of the chest etc) The dog is not known to be normal with the certainty that a man is known to be normal in clinical research.

Knisely A pathologic situation can be defined only as a contrast to a normal one With that in mind, we in our laboratory obtained a series of racing greyhound dogs which were just too slow to win races They were wonderful experimental animals We have gone to all kinds of effort for certain experiments to get healthy animals (Figure 19)

I should like to point out one disadvantage the clinician must face The clinical tests for disease are of such a nature that by definition they are not applied until after the patient says I don t feel very well and comes to the physician So the clinician s concepts of a healthy person are based upon the level of each test above which the patient comes to the clinician By and large, clinicians do not study healthy people at all

Burch That is not correct

Knisely I do not think it is right either but I do think it is true

Burch That is not so For example, normal controls in clinical research often consist of medical students who have had periodic health

FIGURE 19 An example of one of the racing greyhounds used in these experiments.

examinations including questions about their health such as whether or not they are feeling well and whether or not psychologic disturbances exist what they are and the like We can know that several medical students are normal much better than we can know about several mongrel dogs It is the usual practice to assume that dogs are normal

Fine There is one more point I should like to make For our shock experiments we insist on having large, well nourished dogs that weigh a minimum of 30 or 40 pounds It may be that we are just lucky in this respect. At least we used what we thought were well nourished animals We did not use scrawny animals or very young ones Perhaps this kind of selection is not so consistently followed elsewhere for this kind of work.

Burch I think it is important for physiologic research throughout the country that we spend more money supporting animals for research We do not spend nearly enough money for the care of and provision for good animals for this purpose

Fremont Smith We ought to have a veterinary department in every medical school

Burch Those supporting research should be more aware of this problem.

Knisely While we had the racing greyhounds quite unexpectedly a veterinarian, Dr Norman Garlick, came to us and wanted to become a graduate student. He took care of these dogs and they suddenly became more healthy than any dogs I have ever seen They had no lesions on the skin, no hookworms there were no small sores in their ears When Dr Garlick left to go into the army the dogs began to deteriorate immediately In only one week anyone could walk into the dog compounds and on short notice say "There is an animal that is partly sick If we had not seen healthy animals day by day for months we could not have recognized the partly sick ones

Fremont Smith We should not say normal of either animals or human beings unless we add with respect to the following things which were measured Unless we say this, we are giving a completely false impression We should say a man or an animal is normal because he looks well nourished or normal because the following four tests were made

In the case of medical students something else can be said i e. normal with respect to the tests that were made, with respect to the fact that they did not come down with any of the following diseases (and list them in the 4 or 16 months between the time of observation and our point of sending our paper for publication) If there is a subsequent history of failure to come down with illness, it is further indication of the judgment about the person being healthy at the time he was studied which is important and is almost always neglected with respect to this situation.

All this should be written down It will help others to be alert The man who comes down with a serious illness 2 weeks or 3 weeks after

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Burch Our work with veins has shown that the presence or absence of heart worms can produce a difference in certain types of studies

Kinsely I have great respect for the attempt to find healthy human beings using medical students etc. We have done it too But all such attempts today are based on the assumption that a test for disease if negative means health which is not always the case so I think that there must be tests developed for the healthy man which are different from the usual ones in that they are used in the absence of detectable disease

Burch A positive diagnosis of health is made not a negative one.

Kinsely How many men have you tested to see how much work they could do per hour?

Burch That is not necessarily an index of health.

Fremont Smith Yes it is a test of health but it is only one

DeBakey In light of the fact Dr Fine has raised this question of the discrepancy between our results and theirs, it might be desirable for me to comment about it First let me say that we attempted to follow Dr Fine s procedure in carrying out this experiment and we were in communication with him prior to that time and during the experiments so that he was aware of what we were finding We felt that we must be doing something different from the procedure which he was doing in his laboratory since otherwise we should be able to get essentially the same results We were fortunately able to get Dr Frank to go over the experiment with us As Dr Fine has pointed out there were certain things that were different For one thing the animals which we used in general averaged smaller in size than his animals Whether that is a significant difference I do not know but nevertheless that was true We simply were unable to obtain animals of that large size in adequate numbers so that our animals averaged in the neighborhood of 14 or 15 kg

Secondly the temperature in our laboratory was a little higher than the temperature in their laboratory We are acclimated to a little higher temperature than they are in Boston apparently although we had a relatively constant temperature and the laboratory was air-conditioned

As far as the animals themselves were concerned the question has been raised about the possibility that they did develop a resistance I have a feeling that Dr Fine is probably correct about that though we have not checked it as far as our own animals were concerned It is true that our animals were kept in the laboratory for a longer period of time prior to the experiment That was done in part in an attempt

to acclimate the dogs to the laboratory and to feed them if they were undernourished Obviously for this sort of experiment the prolonged stay might have been a bad thing to do

In spite of the properdin levels, these animals did not look like diseased animals They were not scrawny or mangy They had been dewormed However the animals frequently were found to have heart worms at autopsy This is a very common disease among pound animals in our part of the country The number of animals used in these studies was such that the results are statistically significant Rarely did we use less than twenty in a group

As Dr Fine pointed out, the discrepancy between his and our studies was such that we were simply unable to confirm his findings on the protective action of aureomycin. Although we did get some protection it was our feeling that the protection afforded was partial not complete or strikingly good

Shorr Dr Fine reported there was a prolongation of the survival time

DeBakey There was also a slight difference in proportionate number of survivals It was not as statistically significant a difference, but there was a proportionately greater difference

Green Did you use the variable time method Dr Fine uses?

DeBakey We tried to do it exactly the same way

Green Did you find it required a longer time with your treated animals?

DeBakey Yes

Green Was this difference in time more significant than the number of survivals?

DeBakey The number of survivals was the thing we were unable to confirm

Green Was there a significant difference in the time required?

DeBakey Yes

Green Then you have a more striking difference In Dr Fine s method I think you mask the results

DeBakey That may be. Prolongation of time may be the most significant factor

Nickerson Inasmuch as we are discussing antibacterial agents again, I should like to put on record our attempts to duplicate Dr Fine s results with aureomycin. In order to duplicate his procedure as nearly as possible, Dr Lloyd Beck who was working with me at the University of Michigan, visited Dr Fine s laboratory in Boston for a week or so On the basis of his observations we modified our procedure so that it

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was very close to that used in Boston although our dogs averaged slightly smaller than Dr Fine s

When we ran the control and experimental animals simultaneously on adjacent tables to eliminate the effects of variations in temperature, season etc., neither aureomycin pretreatment plus a *priming* dose the day of the experiment nor parenteral aureomycin significantly altered the survival rate, the bleeding volume, or the duration of hypotension. We used both morphine procaine and pentobarbital anesthesia

The role of aureomycin resistant organisms in the failure to demonstrate protection must be considered. We were working in a separate building completely isolated from any hospital connections Penicillin had been used off and on in this laboratory to control infections in animals following operative procedures, but to the best of our knowledge no aureomycin had been used prior to our studies The dogs came from the Detroit pound, were kept in one room for about 2 weeks to rule out distemper or other obvious disease and the aureomycin treatment carried out in a separate room We have no detailed bacteriologic studies on these animals However obvious changes in the character of the feces occurred shortly after the animals were put on aureomycin and the treated animals did not decompose rapidly after death as did the controls The treated animals were almost odorless at post mortem examination even when this was carried out as long as 24 hours after death This was certainly very different from the condition of control animals and strongly suggested to us that the aureomycin had eliminated most of the usual bacteria

Although we did not find any significant effect of aureomycin in our animals I am perfectly willing to grant that bacteria can be a stress We know that bacteria can be the initiating and major cause of shock in both human beings and animals Bacterial action can certainly be one of the factors involved in our experimental shock particularly in cases where there is a considerable period of time for bacterial multiplication between hemorrhage or trauma and death However before we go too far in ascribing specificity to the increased sensitivity to bacteria that Dr Fine has so nicely demonstrated in mildly hemorrhaged animals we should attempt to compare this quantitatively with increased sensitivity to other types of stress

Fine I meant to comment as you requested I should on the point that you just raised We *did test* animals for sensitivity to morphine and found no change whatever *i.e.*, the rabbit 4 hours after transfusion for hemorrhagic shock of 2 hours duration is extremely hypersensitive to endotoxins but the sensitivity to morphine was exactly the same as before shock

Nickerson This is the type of camparison I was referring to but several different tests will be necessary to establish the point Morphine is a test involving central nervous system respiratory depression Perhaps a cardiovascular stress might be more suitable Certainly there are many toxic substances in addition to bacterial toxins to which the previously traumatized animal is more susceptible. The basic principle here is that if you hit a man from two different directions you do not have to hit him as hard each time to get a response.

Dr Haist, will you put on the record your observations and those of Dr H G Downie on the effects of aureomycin in shocked rats?

Haist My observations (31) that aureomycin pretreatment was without benefit in shock produced by a clamping technique, *i.e.*, tourniquet shock, are already on the record from a previous conference But Dr Downie (32), of the Ontario Agricultural College, has performed some experiments on hemorrhagic shock in the rat and found that aureomycin pretreatment in these animals was without beneficial effect also

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INFLUENCE OF ADRENERGIC, CHOLINERGIC, AND GANGLIONIC BLOCKING AGENTS IN EXPERIMENTAL SHOCK*

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THE AGENTS I am about to discuss afforded us another opportunity to test the tenability of our concept of the importance of the ferritin systems in shock. We made no attempt to survey a large number of compounds but instead selected a representative agent from each group for trial The results with dibenzyline will be emphasized, since most of our studies were made with this adrenergic blocking agent

The material presented herein on the influence of these drugs in standardized hemorrhagic and drum shock in rats consists, first of data on survival under various conditions second direct observations of the peripheral vascular pattern in the splanchnic area, and third studies of the hepatic ferritin systems We also carried out studies of their *in vitro* effects on the ferritin systems in normal liver slices The procedures involved are essentially the same as those I described previously (page 103) for our aureomycin studies

The results obtained with dibenzyline in drum trauma are summarized in Table XXX The amounts administered intravenously from 60 to 90 minutes before drumming were 5 μg and 20 μg/100 gm body weight In our experience, the 20 μg dose usually reverses the pressor effect of 0 02 μg of intravenous epinephrine, though the 5 μg dose does not do so Control animals received the same volume of saline as that used to dissolve the dibenzyline The protection afforded by both doses of the drug was substantial

*Aided by research grants from the National Institutes of Health, U S Public Health Service (Grant H 79) the Josiah Macy Jr Foundation the Research and Development Division, Office of the Surgeon General Department of the Army (Contract No DA-49 007-MD-388) the Armour Laboratories and the Postley Fund. The skilled assistance of Iris Forbes, Anne Carleton, and Kurt Weiss in carrying out these studies is gratefully acknowledged Dr Baez is now in the Department of Anesthesiology New York University New York, N Y

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TABLE XXX

Noble-Collip Drum Trauma in Rats After Dibenzyline*

	None	5 µg/100 gm	20 µg/100 gm
Epinephrine response	Pressor	None	Reversal
No of rats	78	56	91
No of rotations	700	700	700
No surviving at 2 hours	43	54	88
No surviving at 24 hours	34	53	87
Per cent survival at 24 hours	43 6	94 6	95 6

Injected 60 to 90 minutes prior to drumming.

In Table XXXI appear the results of experiments demonstrating that the protection afforded by 20 µg dibenzyline against drum trauma could be extended to arenal rats even though it had been shown in our laboratory (1) that such animals are more susceptible to shock The animals were unilaterally nephrectomized 2 or 3 weeks before drumming and a loose ligature of rubberized thread was placed about the opposite renal pedicle the ends being buried subcutaneously On the day of the experiment the ends of the ligature were uncovered under local procaine anesthesia and the renal pedicle was tied just before drumming As many as 81 8 per cent of the dibenzyline injected arenal animals survived as against 20 per cent of their saline-injected arenal controls The marked protection afforded to bilaterally adrenalectomized rats maintained with 1 per cent salt solution by the same dose of dibenzyline (Table XXXII) is still more striking in view of the extreme susceptibility of adrenalectomized rats to drum trauma (2)

I should like now to turn to our data on the comparative action of atropine sulfate and hexamethonium bromide in drum trauma in normal rats shown in Table XXXIII The doses used were 1 mg hexamethonium and 2 mg atropine /100 gm body weight injected intravenously from 30 to 40 minutes before drumming Under the

TABLE XXXI

Noble-Collip Drum Trauma in Arenal Rats After Dibenzyline*

	None	20 μg/100 gm
No of rats	20	22
No of rotations	700	700
No surviving at 2 hours	17	21
No surviving at 24 hours	4	18
Per cent survival at 24 hours	20 0	81.8

*Injected 60 to 90 minutes prior to drumming.

TABLE XXXII

Noble-Collip Drum Trauma in Salt Maintained Adrenalectomised Rats After Dibenzyline*

	None	20 μg/100 gm
No of rats	31	29
No of rotations	600	600
No surviving at 2 hours	4	29
No surviving at 24 hours	0	26
Per cent survival at 24 hours	0	89 6

*Injected 60 to 90 minutes prior to drumming.

TABLE XXXIII

Drum Trauma in the Rat After Pretreatment with Atropine Sulfate* and Hexamethonium Bromide*

	Control Saline	Hexamethonium Bromide 1 mg/100 gm	Atropine Sulfate 2 mg/100 gm
Number of rats	55	26	28
Number of rotations	700	700	700
Number surviving at 24 hours	34	14	22
Per cent survival at 24 hours	62	54	79

Injected I V 30 to 40 minutes prior to drumming.

conditions of our experiment neither one conferred significant protection

Nickerson I should like to comment on the dose of atropine Although it has no adrenergic blocking activity this dose might produce considerable blockade of ganglia if the time intervals were right

Moe Was the hexamethonium doing any good here?

Zweifach Does 1 mg of hexamethonium produce a sustained ganglionic blockade? It was my understanding that such a dose would produce a transitory blockade of perhaps from 30 to 45 minutes at most

Nickerson Yes it would produce some blockade

Moe I do not think there would be very much ganglionic blocking from this dose of atropine In dosage and in duration of ganglionic depression atropine is about equivalent to tetraethylammonium therefore 10 mg /kg of either drug would be required to produce an effect lasting perhaps an hour in the dog or cat I do not know about the rat

Baez In the rat the criteria used to determine the doses to be administered were for the ganglionic blocking agent hexamethonium bromide a reduction of blood pressure of 55 to 60 mm Hg (i e 1 mg) and for the anticholinergic drug atropine sulfate a blocking of the blood pressure lowering action of 10 μg acetylcholine (i e 2 mg) The blocking action lasted over 60 minutes in the rat

Nickerson That would probably be reversed. Acetylcholine would be effective in much lower doses How was the atropine administered?

Baez. Each of the drugs was freshly dissolved in sterile physiologic saline solution and given intravenously in a volume of 0.2 ml. 30 to 40 minutes before the experimental run

In our hemorrhagic procedure, pretreatment with dibenzyline in doses of 5 μg and 20 μg /100 gm body weight was also very effective (3) The survivals, shown in Table XXXIV were 87 5 per cent with the lower and 93 4 per cent with the higher dosage level as compared with only 20 per cent in the controls

The method was the same as that which I described previously graded bleeding controlled after the first hour by a self infusion apparatus The blood pressure was maintained at or above 70 mm Hg for the first hour at 50 mm Hg during the second hour, and at

TABLE XXXIV

The Effects of Dibenzyline* on Graded Hemorrhage in Rats

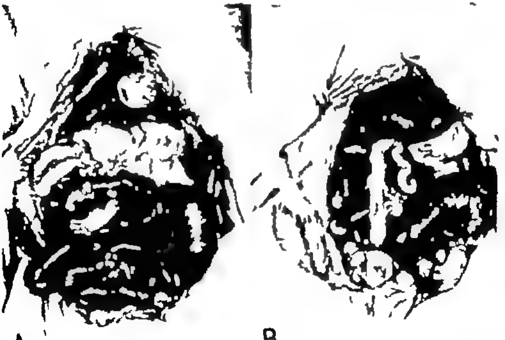
	None	5 μg/100 gm	20 μg/100 gm
Epinephrine response	Pressor	None	Reversal
Number of rats	15	16	15
Maximal blood loss (per cent body wt.)			
Average	3 4	3 5	3 7
Range	2 4 to 4 5	2 3 to 4 4	2 6 to 4.8
Uptake of blood (per cent body wt.)			
Average	0 47	0.28	0 30
Range	0 2 to 0.8	0 0 to 1 0	0 0 to 0 9
Per cent survival at 24 hours	20 0	87 5	93 4

*Injected 60 to 90 minutes prior to bleeding.

30 mm Hg for the remaining 2 hours The amounts of blood shed and later taken up from the reservoir were noted Blood remaining in the reservoir at the end of the 4 hour period was gradually force infused

Congestion of the splanchnic viscera is frequently seen in normal rats subjected to this drastic hemorrhagic procedure or to lethal drum trauma contrasting as mentioned before with the ischemic appearance of the muscle mass and skin The exact mechanisms which govern this pooling of blood in the abdominal viscera are at present obscure The gradual onset of congestion coincides in the hemorrhagic experiment with the spontaneous uptake of blood from the reservoir

Figure 20 is a typical photograph of the viscera of *A* a normal control rat and *B* a rat preinjected with 20 μg of dibenzyline taken after both have been subjected to 700 revolutions in the drum It could serve equally well to illustrate the contrast between control and treated rats after our hemorrhagic procedure Microphotographs of the circulation reflect the gross changes seen here



A B

FIGURE 20 Comparative aspect of abdominal viscera in rats photographed one hour after 700 rotations in the Noble-Collip drum (*A*) control showing congestion and atonic distention of a large portion of the gut and (*B*) dibenzyline pretreated showing normal tonic contraction of the gut and absence of edema and mural hemorrhage

Zweifach When were the lymphatic observations made with respect to the hypotensive sequence?

Baez The mesoappendix microphotographs shown in Figure 21 were taken 60 minutes after a hemorrhagic experiment They are however also typical of the appearance of the terminal vascular bed after drum trauma In the control *A* numerous foci of petechial hemorrhage may be seen while in the dibenzyline treated rat *B* the field appears normal with the integrity of the vascular walls undisturbed In addition we found the metarterioles and precapillary sphincters of the controls to be unresponsive to topically applied epinephrine and to lack vasomotion while those in the treated rats exhibited the usual increased sensitivity and enhanced spontaneous vasomotion characteristic of the compensatory vascular reaction following hemorrhage.

The results of a small series of experiments on the comparative effects of pretreatment with dibenzyline atropine, and hexamethonium in graded hemorrhage in rats are presented in Table XXXV Although in our drum trauma series neither atropine nor hexamethonium had

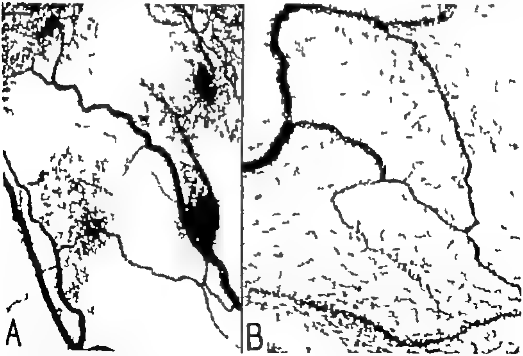


FIGURE 21 Microphotographs of the terminal vascular bed of the rat mesoappendix taken after 4 hours of hemorrhagic hypotension (*A*) control animal showing atonic distention of the collecting venules and petechial hemorrhages and (*B*) dibenzyline-pretreated animal showing tonic contraction of the metarteriole and absence of hemorrhage

TABLE XXXV

Comparative Effect of Dibenzyline* Atropine Sulfate† and Hexamethonium Bromide† in Graded Hemorrhage in the Rat

Pretreatment	Saline Control	Dibenzyline 20 μg/ 100 gm	Atropine Sulfate 2 mg/ 100 gm	Hexamethonium Bromide 1 mg/100 gm
Number of rats	22	19	20	19
Number surviving at 24 hours	6	12	12	7
Per cent survival at 24 hours	27	63	60	37

*Injected 40 to 60 minutes before bleeding.
†Injected 20 to 60 minutes before bleeding

been effective, atropine appeared to offer protection in this hemorrhagic series whereas hexamethonium did not

Burch Was that a very large dose of hexamethonium in respect to the dose of dibenzyline?

Nickerson The major problem in selecting the dose is the duration of action The dibenzyline dose can be regulated quite precisely because this drug produces the same level of blockade for many hours I suspect that this large dose of hexamethonium was given in an attempt to extend the effect over the 4 hour period of stress

Burch Was the dose a single one?

Baez. Yes a single dose

Nickerson At the beginning of the experiment this was a very large dose We do not know the amount of drug present at the end of the hypotensive period because we do not know the rate of renal clearance It would not be a very big dose at the end of the period if the animal were normal

Shorr Do you think the kidney would get rid of very much? Do you think that a shocked kidney would have much of a urine flow?

Nickerson Considerably less than the normal

Remington Do you have any evidence on the degree of ganglionic

blockade effected? Was it sufficient to produce a reduced response to a given nerve stimulation?

Baez. No we have not measured the response to nerve stimulation

Zweifach Can you provide objective evidence that the hexamethonium is actually exerting a blocking action in your experiments?

Baez We have not measured the degree of ganglionic blocking We simply tried the same dose of hexamethonium used by other investigators (4)

Zweifach Effective ganglionic blockade is usually accompanied by a corresponding fall in blood pressure. In instances where the pressure has not fallen or has already returned to normal levels the blocking potentialities of the drug are highly uncertain

Baez. With this dose in the rat we obtained a drop in blood pressure of 50 to 60 mm. Hg However this depressor effect of hexamethonium lasted only from 10 to 12 minutes so that at the time when we began our experiments, the blood pressure was fully restored to control levels The terminal vascular bed however remained plethoric for over 120 minutes

In Figure 22 the data, expressed in per cent of body weight have been averaged for each group of animals in this series The amount of blood shed the duration of maximal bleeding and the rapidity and amount of blood uptake are shown We consider these values to be a reflection of the cardiovascular adjustment during both compensatory and decompensatory phases of the shock syndrome As can be seen the bleeding-out pattern does not differ essentially in the control and experimental groups The peak of maximal bleeding was reached earliest in the atropine-treated rats and last in the dibenzyline treated rats This level was maintained longer by the atropine and dibenzyline groups Impending decompensation indicated by the spontaneous uptake of blood was delayed only in the dibenzyline group The hexamethonium-treated series showed a pattern of bleeding which did not differ from the untreated controls

The status of the hepatic ferritin regulating systems was investigated after drum trauma in a small series of adrenalectomized salt maintained rats The first column in Table XXXVI lists the findings in adrenalectomized rats not subjected to trauma It can be seen in the second column that the liver slices from untreated shocked rats released vasoactive ferritin in oxygen and had completely lost their aerobic ferritin inactivating capacity whereas in the last column, the livers from the dibenzyline treated animals released no ferritin aerobically and were able to inactivate vasoactive ferritin to about the same extent as the adrenalectomized unshocked controls

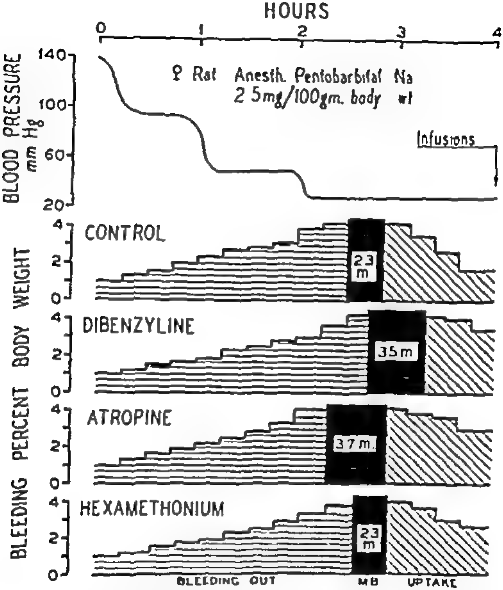


FIGURE 22 Diagrammatic representation of the bleeding pattern in dibenzyline atropine and hexamethonium treated rats and their controls

MB = average maximal blood loss

m = average duration in minutes of the maximal blood loss

Bio-assays of blood samples drawn after drum trauma confirmed the findings in the livers blood from treated animals usually contained no VDM while the opposite was true of the controls

Shorr It is important to note that the rats pretreated with dibenzyline apparently retained their usual ferritin inactivating capacity after

consists of a profound modification of the hepatic ferritin systems It remains to be decided whether this newly described action of dibenzyline would be sufficient in itself to protect these animals against the hypoxia of standard shock procedures by preventing the degree of physiologic deterioration which results in irreversibility

Zweifach Circulatory insufficiency does not develop in the liver of the dibenzyline treated rats?

Baez At present all the agents which beneficially modify the outcome of experimental shock must be administered before the start of the stress procedure Therefore, it would seem that something has been modified to prolong the compensatory adjustments in these animals as compared to their controls

Nickerson I think of this effect of dibenzyline as more the mirror image of your formulation Instead of saying it prolongs compensation I should say that it has prevented something which contributes to deterioration.

Shorr That is correct

Nickerson This simply provides a little different connotation

Shorr Which then requires that we try to establish the nature of the mechanism by which this protection is achieved whether it is the better preservation of the blood flow or whether it is some other effect by which this could be achieved

Horvath Have you measured the blood flow?

Baez We have made direct microscopic observations of the blood flow in the mesoappendix but we have not made any quantitative measurements if that is what you have in mind

Fremont Smith The blood pressure is down in both cases?

Shorr Yes

Fremont Smith So your common denominator is a basic level of blood pressure?

Nickerson And an equal amount of bleeding

Hartt I should like to add here that in the tourniquet type of shock with a 5 hour application period dibenamine which is similar to dibenzyline had a very dramatic effect when given as a pretreatment 20 hours before tourniquet release

Baez We next studied the ferritin oxidation reduction systems as an index of the metabolic state of the liver in rats pretreated with dibenzyline and subjected to hemorrhagic shock (Figure 23) As we demonstrated earlier in the discussion, the liver of untreated shock rats loses both its ability to restrict ferritin release to anaerobiosis and its capacity to inactivate ferritin aerobically In the dibenzyline pretreated rats both of these properties are preserved but this finding does not

tell us whether the protection of the hepatic ferritin systems during the hypoxia of shock is caused by a direct action of the drug on the liver at a cellular level or by a better perfusion of the splanchnic area resulting from the adrenergic blockade.

The striking thing is that dibenzyline permits the hepatic ferritin systems to withstand the hypotensive period without damage (Figure 23)

The possibility of a direct effect of these agents upon liver metabolism, as contrasted with an indirect effect brought about by better perfusion of the splanchnic area, was now investigated by *in vitro* exposure of normal liver slices to the drugs in the same manner as I described earlier for our aureomycin *in vitro* studies (page 103)

In each instance liver slices obtained from the same normal rat were exposed for 10 minutes under aerobic conditions, to dibenzyline, atropine, or hexamethonium in respective concentrations of 2 µg 20 µg and 10 µg/gm of tissue. The slices were then washed

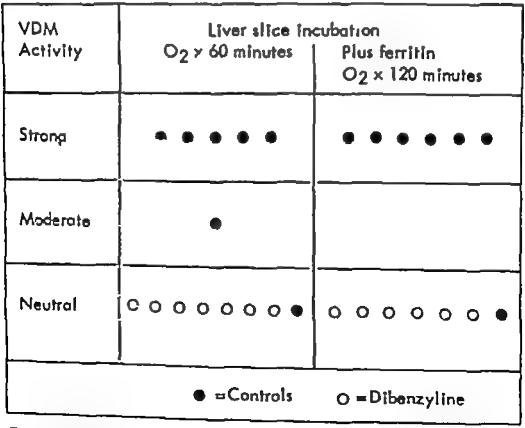


FIGURE 23 Effect of pretreatment with dibenzyline (20 µg/100 gm. body weight injected from 60 to 90 minutes before bleeding) on the hepatic ferritin systems after hemorrhagic hypotension.

and incubated in nitrogen for 90 minutes for tests of anaerobic ferritin formation. Following this exposure to anaerobiosis the aerobic ferritin inactivation capacity of the slices was tested. The controls consisted of slices from the same liver treated similarly except for the omission of the drug. The details of the procedure are the same as described earlier in the discussion (page 103).

The dibenzyline part of the experiment illustrated in Figure 21 is the important part. At this low concentration it completely prevented the anaerobic release of ferritin and also preserved the ferritin inactivation mechanism from deterioration during the 90-minute anaerobic period. The control liver slices obtained from the same rat invariably showed anaerobic production of vasoactive ferritin and a total loss of ferritin inactivation capacity.

Zweifach: What method is being used to determine the ferritin content?

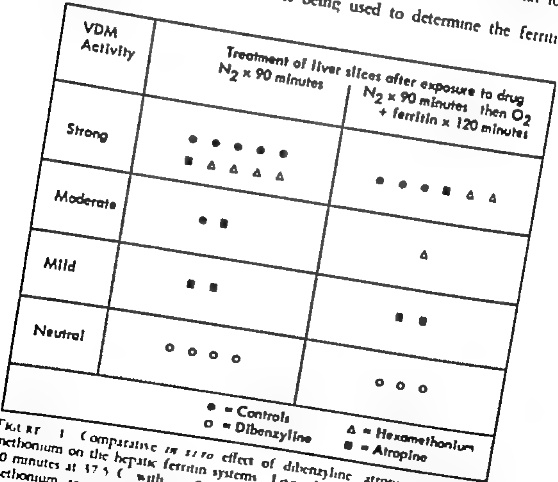


FIGURE 21. Comparative *in vitro* effect of dibenzyline, atropine and hexamethonium on the hepatic ferritin systems. Liver slices (1 gm.) incubated for 10 minutes at 37.5 C. with 0 and 10 μg. dibenzyline, atropine and hexamethonium, respectively, in 5 ml. Ringer phosphate medium in oxygen. Slices washed twice and reincubated

Baez. We use the rat mesoappendix assay to determine the VDM activity of a given sample of crystalline ferritin

Engel What does dibenzyline alone do when applied directly in the mesoappendix preparation?

Baez. Dibenzyline induces in the normal rat a marked decrease in the sensitivity of the metarterioles and precapillary sphincters to topically applied epinephrine and a suppression of vasomotion (5 6) The intensity and duration of this effect are directly related to the amount given However after 1 8 to 2 per cent of bleeding the rat injected with dibenzyline exhibits an over all pattern of compensatory adjustment in the peripheral vascular bed equal to that seen in unin jected control rats after the same amount of bleeding

Shorr Dr Engel s question was whether or not the medium after this treatment contained enough dibenzyline to influence the bio-assay

Baez. I believe not. The amount of dibenzyline used was 2 μg /gm of tissue. After the 10-minute oxygen exposure, the medium con taining the drug was discarded and the slices were washed twice before they were incubated in nitrogen. The second wash was tested and found to be vasoinert

Nickerson There is an added safety factor If any appreciable time elapsed between the incubation and the mesoappendix assay any residual dibenzyline in the medium would have decomposed

Shorr The intervals would be 90 minutes in the one, and 90 plus 120 minutes in the other

Baez. In this case we are getting neutral assays if there were significant dibenzyline contamination we would expect VDM effects

Engel The implication of your study is that the dibenzyline does have a direct effect on liver metabolism. It presumably must have a rather broad effect since there is at least one other report (7) from Canada which shows the rise in plasma amino nitrogen during hemor rhage to be less in dibenzyline treated animals than in untreated animals

Horvath There are several other points too The volume of blood they withdrew from dibenzyline treated animals was considerably less than from those animals which were not pretreated with dibenzyline. If the dibenzyline was given 85 minutes or so after the bleeding it was not effective. In other words there was a decided time element as to when the dibenzyline produced an effect If it was given too late, it did not have any effect. There was a certain critical time in which the dibenzyline produced this beneficial effect which was related to the blood volume

Nickerson For the time intervals to mean anything the shock procedure as well as the time of drug administration must be specified

and incubated in nitrogen for 90 minutes for tests of anaerobic ferritin formation. Following this exposure to anaerobiosis the aerobic ferritin inactivation capacity of the slices was tested. The controls consisted of slices from the same liver treated similarly except for the omission of the drug. The details of the procedure are the same as described earlier in the discussion (page 103).

The dibenzyline part of the experiment illustrated in Figure 24 is the important part. At this low concentration it completely prevented the anaerobic release of ferritin and also preserved the ferritin inactivation mechanism from deterioration during the 90-minute anaerobic period. The control liver slices obtained from the same rat invariably showed anaerobic production of vasoactive ferritin and a total loss of ferritin inactivation capacity.

Zweifach: What method is being used to determine the ferritin content?

VDM Activity	Treatment of liver slices after exposure to drug: $N_2 \times 90$ minutes	Treatment of liver slices after exposure to drug: $N_2 \times 90$ minutes, then O_2 + ferritin $\times$ 120 minutes
Strong	● ● ● ● ● ■ △ △ △ △	● ● ● ■ △ △
Moderate	● ■	△
Mild	■ ■	■ ■
Neutral	○ ○ ○ ○	○ ○ ○

● = Controls　○ = Dibenzyline　△ = Hexamethonium　■ = Atropine

FIGURE 24 Comparative *in vitro* effect of dibenzyline, atropine and hexamethonium on the hepatic ferritin systems. Liver slices (1 gm.) incubated for 10 minutes at 37.5 C with 2, 20 and 10 μg dibenzyline, atropine and hexamethonium, respectively, in 5 ml Ringer phosphate medium in oxygen. Slices washed twice and reincubated.

Baez. We use the rat mesoappendix assay to determine the VDM activity of a given sample of crystalline ferritin.

Engel What does dibenzyline alone do when applied directly in the mesoappendix preparation?

Baez Dibenzyline induces in the normal rat a marked decrease in the sensitivity of the metarterioles and precapillary sphincters to topically applied epinephrine and a suppression of vasomotion (5 6) The intensity and duration of this effect are directly related to the amount given. However after 1.8 to 2 per cent of bleeding the rat injected with dibenzyline exhibits an over all pattern of compensatory adjustment in the peripheral vascular bed equal to that seen in unin jected control rats after the same amount of bleeding

Shorr Dr Engel s question was whether or not the medium after this treatment contained enough dibenzyline to influence the bio-assay

Baez I believe not. The amount of dibenzyline used was 2 μg /gm of tissue After the 10-minute oxygen exposure the medium containing the drug was discarded and the slices were washed twice before they were incubated in nitrogen The second wash was tested and found to be vasoinert.

Nickerson There is an added safety factor If any appreciable time elapsed between the incubation and the mesoappendix assay any residual dibenzyline in the medium would have decomposed

Shorr The intervals would be 90 minutes in the one, and 90 plus 120 minutes in the other

Baez. In this case we are getting neutral assays if there were significant dibenzyline contamination we would expect VDM effects

Engel The implication of your study is that the dibenzyline does have a direct effect on liver metabolism. It presumably must have a rather broad effect since there is at least one other report (7) from Canada which shows the rise in plasma amino nitrogen during hemor rhage to be less in dibenzyline treated animals than in untreated animals

Horvath There are several other points too The volume of blood they withdrew from dibenzyline treated animals was considerably less than from those animals which were not pretreated with dibenzyline. If the dibenzyline was given 85 minutes or so after the bleeding it was not effective In other words there was a decided time element as to when the dibenzyline produced an effect. If it was given too late it did not have any effect There was a certain critical time in which the dibenzyline produced this beneficial effect which was related to the blood volume

Nickerson For the time intervals to mean anything the shock procedure as well as the time of drug administration must be specified

Administration of dibenzyline 85 minutes after bleeding was actually treatment after the shock had become irreversible because their total shock period was only 90 minutes They found that dibenzyline given 30 minutes after the induction of hypotension still protected

There are several factors of interest in relation to the dibenzyline protection One is that dibenzyline reacts very rapidly and preferentially with sulfhydryl compounds (8) I am not sure that the present data are adequate to show that the dibenzyline protection is brought about by an action on a metabolic system in the liver as distinct from the vascular effects of adrenergic blockade. However the tools necessary to decide this point are available Many compounds are known which have the same reactive alkylhalide grouping as dibenzyline, and which react with sulfhydryl groups in essentially the same way, but which do not block sympathetic activity It will be important to test such compounds for their ability to protect against shock and to protect the liver against the effects of anaerobiosis

Engel Did I understand correctly that you gave your animals 20 μg /100 gm and you also added 20 μg to however much you had in the vessel in which the liver was incubated?

Baez. In the shock experiments in which the livers were removed for study after the hemorrhagic procedure 20 μg /100 gm body weight of dibenzyline had been injected intravenously 60 minutes before the experimental run and no dibenzyline was added to the liver slices for incubation In the *in vitro* experiments normal liver slices were exposed for 10 minutes to 2 μg of the drug/gm of tissue in a volume of 5 ml This solution was discarded and the slices were washed twice before they were incubated in nitrogen

Engel Presumably one to 2 μg would still be a lot more than the liver would get from the injection into the animal

Baez. You may be right I would not want to guess how much of the dibenzyline injected *in vivo* would reach the liver We have really not explored the minimal dose capable of effectively blocking the anaerobic release of ferritin *in vitro* This should be studied

I should like to say in conclusion that I believe we have clearly demonstrated an action of dibenzyline on the hepatic ferritin systems which is independent of its adrenergic blocking properties However the data I have presented permit no inferences regarding the extent to which each of these two actions enters into the protection afforded by this agent in shock

Frank Do you have an explanation for the very similar action of aureomycin and dibenzyline in this respect?

Baez. The same effect is brought about but at present we do not understand the mechanism.

Frank Do you think it is a common pathway for the two molecules?

Baez I am not in a position to answer your question Although the end result is the same, we have no specific information at present on the pathway of either molecule

Shorr According to the concept which we are using as a working hypothesis this agent affects the liver ferritin mechanisms in a favorable manner On the other hand, the perfusion of the splanchnic organs is also favorably influenced by the reduction of vasoconstriction which this drug brings about As long as there are two factors operating it is impossible to decide which is of crucial importance for recovery Similarly with aureomycin there is the problem of its antibiotic action and its action on these same systems What we require therefore is a compound which has no pharmacologic action and which yet retains the same beneficial influence on the ferritin systems. Such a compound should help to decide whether or not the ferritin systems play a significant role in shock. We have some new evidence to present on this point later

Nickerson Information on the role of vascular factors in the protection provided by adrenergic blocking agents can also be obtained from experiments involving adrenergic vasoconstriction. We have done some work on the production of shock by vasoconstriction and were surprised to find how sensitive animals are to its deleterious effects Infusion of norepinephrine into dogs at a rate of only 2 0 μg /kg /min (base) will produce typical irreversible shock in a high percentage of the animals within less than 4 hours

Fremont Smith Is this the same kind of thing Freeman did many years ago with ordinary epinephrine?

Nickerson Freeman (9) found that epinephrine infusions caused marked reductions in circulating blood volume Erlanger and Gasser (10) had previously shown that infusions of epinephrine could produce death from what appeared to be shock, but in their experiments the epinephrine was infused in large amounts until the circulation failed and the animal died Most of our animals die long after the end of the infusion The course of the blood pressure in these experiments is of some interest In a typical experiment it may increase from a mean of 130 mm Hg to 180 mm. Hg during the first 10 or 15 minutes of infusion and then gradually return to normal during the remainder of the infusion period At the end of the infusion the animal is essentially normotensive although this does not mean that vasoconstriction is not present The cardiac output is much below the control level

Zweifach. Do these animals show signs of pulmonary edema or any evidence of reduced oxygen tension in the bloodstream?

Nickerson. No obvious pulmonary edema. After termination of the infusion, many animals recover from the light barbiturate anesthesia and appear to be quite alert and active. Most deaths occur from 12 to 24 hours after termination of the infusion.

Dobson. What kind of animals did you use?

Nickerson. Dogs.

Levine. In the adrenalectomized animal there is the same result but much earlier and in response to smaller amounts.

Shorr. Is this in the rat or the dog?

Levine. In the rat.

Nickerson. Your results fit into the general pattern of greater sensitivity of adrenalectomized animals to many forms of stress.

Horvath. Would hypertension occur with neosynephrine instead of epinephrine?

Nickerson. My guess is that it would, but I know of no data on which to base a definite answer.

Horvath. It does not, though the pattern is the same. If neosynephrine is given, an increase in blood pressure is obtained, and as time goes on and infusion continues the blood pressure has a tendency to fall. These animals will not go into shock. They can die afterward.

Nickerson. What is the duration of the infusion?

Horvath. It is 3 or 4 hours.

Burton. I should like to go back to the experiments with the blocking agents. Most others find that when a blocking agent is used less bleeding volume is needed to lower the pressure. In the subsequent time there is a greater self infusion to keep the animal at that pressure than there is in the unblocked animal. But I gathered that this was not the finding in these experiments. Did you not find a much lower bleeding volume and a greater reinfusion? Isn't this a strange discrepancy from the work of several other groups?

Baez. You are correct that there is a discrepancy in the pattern of bleeding as reported by others. In our Figure 22 and Table XXXV an equal or larger volume of blood than in controls has been shed by the dibenzyline treated group. This was also the case in dogs in our laboratory (11). However, the method of graded bleeding we used in these experiments is probably responsible for this difference. When hypotension is rapidly induced the animal may be deprived of the complete compensatory response of which his circulatory system is capable. In our hands the significant difference in the bleeding pattern between the treated and the untreated rats is a delay in spontaneous

blood uptake by the treated animals and as you pointed out, a slightly larger volume of blood remains to be reinfused at the end of the experimental period

Horvath Some of the other reports also have variable figures They come in and state the converse the volume of blood is reduced.

Nickerson I should like to quote Dr Burton some data from the University of Western Ontario I believe that in his thesis Dr Lloyd Beck, now at the University of Michigan reported that even with his fairly rapid bleeding total bleeding volumes were statistically indistinguishable in the control and dibenamine treated groups if the dose of blocking agent was relatively small. Protection is also obtained with large doses of blocking agent but the animals bleed less The dose of blocking agent given is very important If compensatory mechanisms are completely abolished and some drug toxicity is added to the stress of bleeding, the total bleeding volume may be reduced However, there is a range of blockade in which the blood loss is equal to that of the controls and protection is still demonstrable Another factor of importance is the rapidity of bleeding Dibenzyline- or dibenamine treated animals do not compensate by reducing the volume of their vascular system as rapidly as untreated animals They can ultimately adjust to the decreased volume, but the response is slower

Frank I should like to agree to the two points that have been made, the rate of bleeding and the dose. Also there may be a species difference as well because in our experience with rapid bleeding as much blood was obtained from dibenaminized rats as from untreated rats Instead of setting the duration of hypotension arbitrarily we waited for spontaneous return of blood from the reservoir Our dibenaminized rats in 8 hours had not taken back as much blood as the control rats did in 3 or 4 hours, and about 70 or 80 per cent of the treated ones survived whereas only 10 or 15 per cent of the untreated survived So in the rat the benefit seemed unequivocal

In our dog experiments if we gave enough dibenamine to get clear-cut epinephrine reversal, we never could bleed our dibenaminized dogs nearly to the extent that control dogs would bleed We had the strong impression the only protection we were conferring was by virtue of the lesser degree of reduction of blood volume

Burton They also took back more didn t they?

Frank Yes

Baez. In the dog experiments I mentioned before, we gave 2 mg of dibenzyline/kg of body weight from 15 to 18 hours before bleeding at which time the pressor effect of epinephrine was still reversed Dr

Frank would you elaborate a bit on your dibenzyline dosage and time schedule?

Frank We gave three dosage programs to the dogs some 5 mg /kg 0 5 to 1 hr before shock 15 mg /kg at the same interval before shock, or 20 mg /kg 18 to 20 hours before shock. It was only after the small dose which produced epinephrine blockade but not reversal that the animals bled normally Dr Remington (12) published a similar experience some years ago

Remington We seem to be repeating what was said 5 years ago It was then pointed out that the effect on lethal bleeding volume was related to the dosage level of dibenamine used May I also remind you that the blood flow through the gut in the dibenaminized dog falls as low no matter what dose or what bleeding volume was used to reduce the pressure to the same low level as in the control animal The amount of oxygen extraction in the two animals cannot be distinguished Third may I still strongly champion the idea expressed then that the bleeding volume is not necessarily a proper means of evaluating the amount of circulatory stress The heavily dibenaminized dog is trying to perfuse a whole vascular system A relatively small blood loss may cause a large pressure fall Despite the low bleeding volume, his circulation may be just as precariously balanced as that of a control animal who can effectively shut off large areas of its original vascular bed and requires a larger bleeding volume to effect the same pressure fall

Nixon I think it is important though to recognize that they can and still have a different survival

Nickerson The difference between the ease with which dibenzyline protection is demonstrated in the rat and the dog probably is an expression of a fundamental difference between these two species The rat perhaps because of its size or its native toughness has a larger margin of safety than the dog The dose of blocking agent must be just right in order to obtain both protection and equal bleeding in the dog The rat has a fairly wide range within which this is possible and everyone seems to get comparable results

Frank There is another difference it is very obvious and it relates to what you spoke of earlier the fact that the blood pressure level of 30 or 35 mm Hg in a dog is very critical and if it drops a millimeter or two below that the dog stiffens with permanent central nervous system damage If arterial pressure is kept above that the dog is all right

Our experience with rats is that very often a rat cannot be bled abruptly down to anywhere near that low a blood pressure without its becoming apneic we have been forced to give back blood and bleed more gradually in the case of a control rat The rat seems to vasocon

strict more strongly than the dog because you can just about exsanguinate some rats at a blood pressure of 100 mm Hg However dibenaminized rats can be bled smoothly without their experiencing respiratory difficulty

Nickerson Does anyone know whether the rat has more sympathetic vasoconstrictor control in its cerebral vascular bed?

Frank The rat acts as though it does. I wonder if someone can answer that. Certainly his brain stem response seems different.

Furchgott I should like to ask a question about the *in vitro* experiments Granted that most of the dibenzyline has been removed by washing at the end of the exposure period, and that a good part of the dibenzyline remaining in the tissue would undergo degradation during the course of the incubation, is there not yet the possibility that there might still be sufficient dibenzyline present during the course of the incubation to react with the SH groups of the ferritin released and thus inactivate ferritin?

Baez I really cannot guess the amount of dibenzyline necessary for this reaction. However we were not able to detect any change in the VDM activity of (0 0008 μg N) crystalline ferritin on incubation with arbitrarily chosen amounts of dibenzyline (0 01 and 0 1 μg) which *per se* are vasoinert. Consequently I am inclined to believe that the dibenzyline is not interacting directly with the ferritin but that it requires an intact cell or cell product for this effect

Furchgott In the control experiments in which a mixture of dibenzyline and ferritin is tested should the injection be made immediately after mixing or should an incubation period be allowed before injection?

Baez The mixtures were incubated in the room air for 20 minutes before bio-assay

Furchgott You never incubate an hour at 37°C.

Baez No

Shorr I think Dr Furchgott s point is this Should we have incubated 90 minutes?

Baez We have not done that. In one trial the VDM effect was not destroyed when ferritin and dibenzyline were incubated in oxygen for 20 minutes

Horvath Did you incubate at room temperature of 28° or 25°C or whatever the room was or at 37°C.? That amount of temperature difference makes a great deal of difference in response of even the same reactions or chemical reactions involved

Baez Both the oxygen and the air incubations were at 37 5°C. What I meant was that in addition to air we also tried an environment of oxygen for 20 minutes

Shorr: At 37.5°C.

Horvath: Why do people keep on using 37°C. when that is a very illogical sort of temperature?

Shorr: What is more logical?

Horvath: Is that the temperature of the tissue you are studying?

Shorr: Can you suggest a more logical temperature?

Horvath: I think so.

Shorr: I should not think the temperature would be so critical that 1 or 2 degrees would make any difference. Performing all the metabolic experiments at 37.5°C. is certainly better than having the temperature uncontrolled.

Horvath: The temperature varies considerably.

Shorr: How wide are the variations?

Horvath: As much as 10 or 20 degrees.

Shorr: Ten or 20 degrees centigrade?

Horvath: Yes.

Nickerson: Not the deep tissues.

Horvath: What do you mean by deep tissues? The liver can be 40° or 41°C.

Nickerson: But not 10 degrees from 37.5°C.

Horvath: Other tissues may have temperatures of about 20°C.

Shorr: Do you think this is a serious objection?

Horvath: No. I was just curious why people use 37°C., and I have never been able to find anybody able to tell me anything except that it is convenient.

Fremont-Smith: And conventional.

Horvath: Convenient and conventional are tied in together.

Shorr: We now have many facts collected at 37.5°C. which are comparable in this respect and this is one of the compelling reasons why we continue to use it.

Fremont-Smith: You are making the point, not comparable in the body necessarily, although it is comparable outside the body. We are making a tacit assumption that it is comparable within the body. We must watch out for that. Isn't that your point?

Horvath: Yes.

Shorr: We could only wish that other conditions of our experiments were not less comparable to those in the body.

Levine: Ours was a backdoor approach to shock because the experiments* which were performed between 1950 and 1952 were not done

*The experiments here reported consist of the work of Dr. Maurice Goldstein, Dr. Estelle Ramey, and Dr. Irving Fritz, who were jointly responsible with me for the work and ideas developed.

for the primary purpose of a study of shock. We wanted to study the mode of action of the corticoids on stress, and we selected muscular exercise as the stress which could be most conveniently observed Muscular exercise in the adrenalectomized animal leads to very rapid fatigue and if continued beyond the stage of fatigue ends in death caused by vascular collapse

The first thing which we asked ourselves was, did the susceptibility to fatigue denote any peculiar metabolic changes or differences from normal in the neuromuscular or muscular apparatus of the adrenalectomized animal?

Figure 25 shows the contraction of a rat diaphragm stimulated via the phrenic nerve. It can be seen that the diaphragm taken from the adrenalectomized animal contracts as vigorously and for as long a period as the same muscle taken from a normal animal Even when taken out of adrenalectomized animals which were exhausted *in vivo* (by swimming) the diaphragm performs normally *in vitro*

Many such experiments convinced us that the fatigability of the adrenalectomized animal *in vivo* was not caused by intrinsic functional impairment of nerve, neuromuscular junction or even the muscle itself (13-18)

In experiments with dogs the procedure was to stimulate the gastrocnemius muscle *in situ* at the rate of three stimuli per minute

In the normal animal, the contraction, even after 12 hours remained steadily at its initial height. The blood pressure was not significantly changed throughout this period (Figure 26)

In the adrenalectomized animals the contractions again were initially as vigorous as in the normal group and continued about 2 to 3½ hours Then the contractions began to fail and disappear almost completely but invariably this was preceded by a fall in the blood pressure and by other criteria signifying shock, such as hemoconcentration, cardiac output changes etc When the blood pressure fell below 70 mm Hg there was failure of the contraction (Figure 27) If stimulation is continued beyond this point, the blood pressure will continue to fall and the animal will die during the next hour

We wanted to demonstrate that if the blood pressure could be artificially raised then the contractions would resume and become forceful That can be done temporarily as the blood pressure is raised, contraction revives again only to fall back once more Among other devices we used norepinephrine, and we found that one must use very large doses of norepinephrine during the period of stress in the adrenalectomized animal, to raise its blood pressure, amounts far greater than are needed in the normal animal

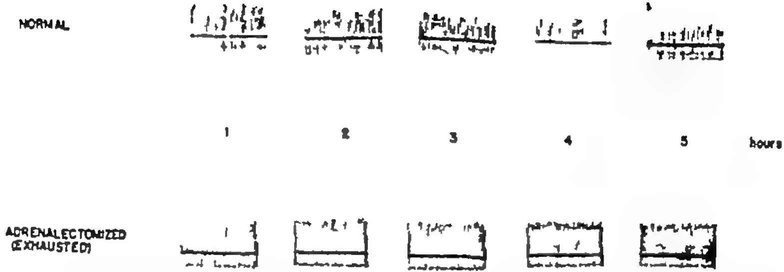


FIGURE 5. *In vitro* contraction of rat diaphragm stimulated via phrenic nerve. Body weight 200 gm. stimulated 6/min. Reprinted, by permission, from Ramey, E. Goldstein, M. S., and Levine, R. Mechanism of muscular fatigue in adrenalectomized animals. *Am. J. Physiol.* 162, 10 (1950).

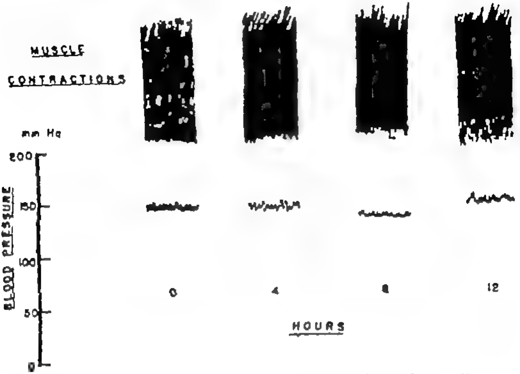


FIGURE 26. Contraction of dog gastrocnemius *in situ* (normal animal) stimulated 3/min. Reprinted, by permission, from Goldstein, M. S., Ramey E. R., and Levine, R. Relation of muscular fatigue in the adrenalectomized dog to inadequate circulatory adjustment. *Am J Physiol 163, 561 (1950)*

Burch Did the decrease in blood pressure cause the muscle fatigue?

Levine Yes when the blood pressure drops below 70 mm. Hg the muscle begins to show significant fatigue

Green Did I understand correctly that there was hemoconcentration in the dog before the blood pressure fall?

Levine Yes there was hemoconcentration at the time of this fall

Burch Did fluid enter the contracting muscles?

Levine I cannot say I do not think so as far as sizeable edema is concerned It did not swell visibly

Furchgott May I ask whether this is continuous stimulation?

Levine Continuous over the whole period Only portions of the recordings are shown in Figures 26 and 27 Next we compared the normal with the adrenalectomized dog when both were given three doses of norepinephrine intravenously (0 25 0 5 and 10 μg/kg) The pressure rises were proportional to the dose in the normal dog However in the adrenalectomized animal under anesthesia which is sufficient stress the blood pressure effect is distinctly diminished (Figure 28) *

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* For nor-adrenaline read norepinephrine.

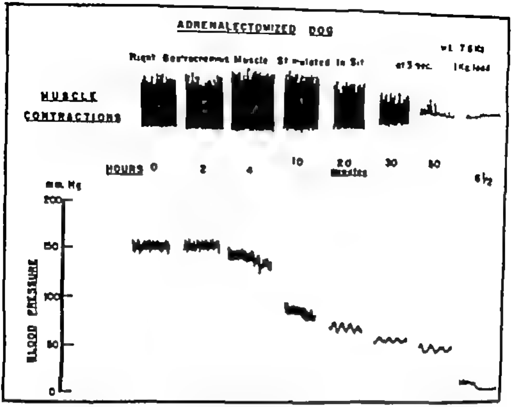

FIGURE 27 Contraction of dog gastrocnemius adrenalectomized animal Reprinted by permission from Goldstein, M S., Ramey E. R. and Levine, R. Relation of muscular fatigue in the adrenalectomized dog to inadequate circulatory adjustment *Am J Physiol* 163 561 (1950)

Figure 29 demonstrates again the relative insensitivity to norepinephrine which develops during stress in the adrenalectomized dog Initially the effect of a norepinephrine dose is not too different from the normal control But as the animal stays under the influence of the anesthetic for from 40 to 60 minutes the blood pressure falls and the response to norepinephrine becomes more and more feeble This occurred with every type of stress employed in the adrenalectomized animal

If normal dogs are given a constant intravenous infusion of norepinephrine (Figure 30) the blood pressure rise occurs immediately and the high level is maintained If one discontinues the infusion the blood pressure falls if more of the drug is given the blood pressure rises again However in the adrenalectomized animal the norepinephrine itself seems to exert a stress because it does not raise the blood pressure as well as it does in the normal animal and also because irrespective of continuous infusion the blood pressure begins to fall

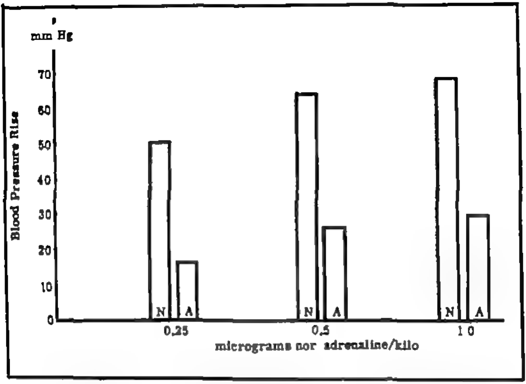

FIGURE 28 Comparison of blood pressure rise in the normal (N) and the adrenalectomized (A) dog Reprinted, by permission, from Ramey E. R. Goldstein, M S. and Levine, R. Action of nor-epinephrine and adrenal cortical steroids on blood pressure and work performance of adrenalectomized dogs. *Am J Physiol* 165 450 (1951)

More norepinephrine will raise the blood pressure temporarily Despite trebling the dose of the drug stress continues until death in shock.

We were still concerned, of course with the mechanism of stress in the adrenalectomized animal the end result of which is a shock state We felt that we needed some working hypothesis with which to operate. The working hypothesis which we developed was outlined as follows If in the normal animal a stress is induced be it bleeding muscular exercise formalin injection or cold exposure this stress will be signaled to many tissues Let us consider the signals to the central nervous system and the responses elicited It is agreed that the pituitary is activated, leading to adrenal cortical activity and an outflow of cortical steroids Suppose that in addition there is also an activation of the autonomic nervous system centers leading to a greater output of effector substances such as norepinephrine epinephrine acetylcholine etc. It is known that when the adrenal cortex is removed the same stress seems to have a more deleterious effect than it does if the adrenal cortex is present.

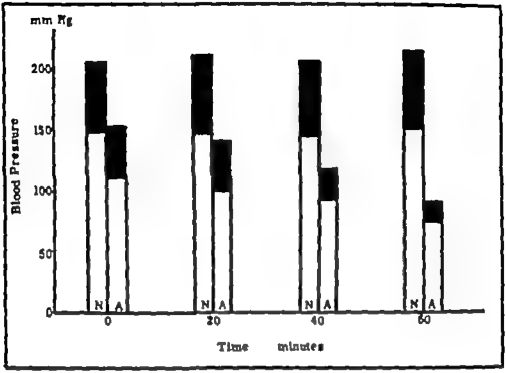

FIGURE 29 Development of insensitivity to norepinephrine during stress of the adrenalectomized dog N = normal A = adrenalectomized. White bar = initial pressure black bar = rise above initial pressure Reprinted by permission, from Ramey E. R., Goldstein, M. S., and Levine R Action of nor-epinephrine and adrenal cortical steroids on blood pressure and work performance of adrenalectomized dogs. *Am J Physiol* 165 450 (1951)

Our assumption was that in the absence of steroids the compensation induced by the autonomic nervous system may become harmful If that were a correct view we reasoned that in the adrenalectomized animal blockade of the autonomic system might induce a new type of balance in which survival would be possible This reasoning led us to use certain blocking agents

Figure 31* shows an experiment on an untreated bilaterally adrenalectomized dog maintained on desoxycorticosterone but not receiving 17-OH corticoids He was bled at the times indicated by the arrows and at every point a portion of blood was removed the total of which amounted to between 8 and 12 ml /kg There was poor capacity to readjust the blood pressure after every bleeding episode

When the blood was reinfused there was some blood pressure maintenance at a level between 50 and 60 mm Hg but in such animals the reinfusion did not restore normal pressures In the normal animal

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* [illegible]

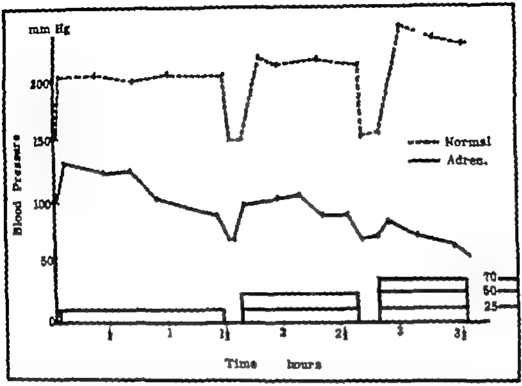

FIGURE 30 Rise and fall of blood pressure with constant intravenous infusion of norepinephrine in the normal and in the adrenalectomized dog Reprinted, by permission, from Ramey E. R., Goldstein M. S., and Levine, R. Action of nor epinephrine and adrenal cortical steroids on blood pressure and work performance of adrenalectomized dogs. *Am J Physiol* 165 450 (1951)

the same procedure, with the same amount of blood withdrawn and reinfused would compensate completely This is not the stage of irreversibility in the normal dog but it is irreversible in the adrena lectomized dog

However if the adrenalectomized dog was pretreated with banthine in dosages sufficient to block the ganglia, and then was given some additional banthine during the procedure the same amount of bleeding produced similar reactions during the withdrawal When the total amount of blood was reinfused the changes were those seen in the normal animal The blood pressure was restored to prebleeding levels and the animal survived Evidence for the ganglionic blockade was the observation that stimulation of the sciatic or stripping of the gut did not induce any changes in blood pressure

Therefore, banthine in this dosage, has a double effect. It would in this dosage of course inhibit the cholinergic effectors but at the same time it is a ganglionic blocking agent

Figure 32 shows a different type of stress namely muscle work The

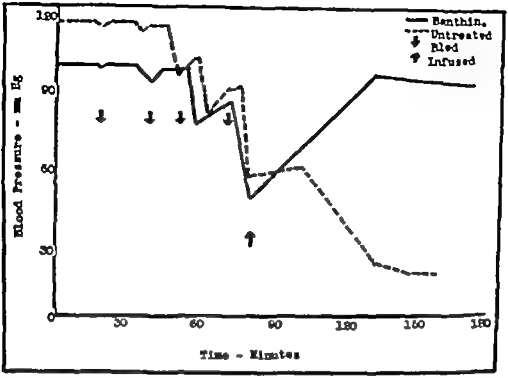

FIGURE 31 Blood pressure responses in untreated and in banthine pretreated bilaterally adrenalectomized dog maintained on desoxycorticosterone but deprived of 17-OH corticoids. Reprinted by permission from Goldstein, M S., Ramey E R. Fritz, I and Levine R. Reversal of effects of stress in adrenalectomized animals by autonomic blocking agents use of atropine banthine and dibenamine. *Am J Physiol* 171 92 (1952)

graph shows the force of contraction in terms of the per cent of the height of the initial contraction The blood pressure record is also shown It is evident that the blood pressure falls before contractions are affected The untreated adrenalectomized animal dies in shock as shown

However banthine protected such animals against the effect of the muscular contraction on vascular regulation The blood pressure was maintained and so was the height of the initial contraction

The same favorable results can be obtained in the adrenalectomized animal if it is given C 17 OH steroids before the experiment It would seem then that the 17 OH steroids protect the animal in some way from the deleterious effects of autonomic overactivity Atropine also protected the animal against the effects of induced hemorrhage

Nickerson What was the dose of atropine?

Levine The dose of atropine was 2 mg to the animal as a whole at the time of induction of anesthesia The dose of banthine was

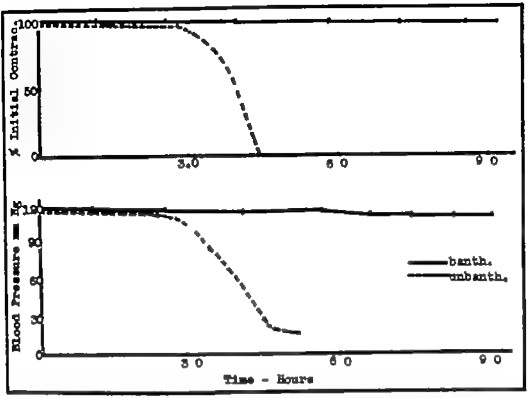

FIGURE 32 Muscle stress—effect of repeated muscle contractions upon the force of contraction and upon the blood pressure in treated and untreated adrenalectomized animals. Reprinted, by permission, from Goldstein, M. S., Ramey E. R., Fritz, I. and Levine, R. Reversal of effects of stress in adrenalectomized animals by autonomic blocking agents use of atropine, banthine and dibenamine. *Am J Physiol* 171 *92 (1952)*

3 mg to 5 mg/kg, 30 minutes before the experiment and an additional 3 mg/hour to the whole animal during the experiment

Banthine protected the adrenalectomized rat against a formalin stress as measured by survival and the observation of blood flow in the mesoappendix The results were the same as those shown by Dr Baez in his dibenzyline experiments

Norepinephrine given in large doses to the adrenalectomized animal, induces the deleterious picture observed during shock in the mesoappendix When a blocking agent like dibenzyline is given the blood flow pattern remains normal

Increased autonomic activity is beneficial for the body if it acts at the same time in the presence of adrenal steroids but may become deleterious if it occurs in the absence of the steroids I am wondering whether in the normal animal in which a shock procedure is induced, the effects of the blocking agent are also to mask a certain amount of this deleterious effect of overregulation We have not gone more deeply

into this phase because as I pointed out at the beginning we did not get into this field from the standpoint of studying shock and we did not continue these studies

One consequence has been a clinical one which is very difficult to evaluate In clinical shock norepinephrine is frequently used The amount of norepinephrine needed in order to bring the blood pressure from shock levels to about 100 mm. Hg may be enormous We wondered whether under those circumstances the addition of steroids would help sensitize the organism to smaller amounts of the constrictor agent

Scientific evaluation of results is practically impossible because of absence of control data but it would appear that steroids have helped, under certain circumstances, to sensitize the human in shock to norepinephrine That impression is shared by a few others

Frank Which steroids?

Levine Hydrocortisone

Nickerson You are talking about autonomic overactivity as if it were a single entity Actually when the autonomic system is activated reflexly by stress drugs or some other factor the two divisions tend to respond in opposite directions *i.e* the activity of one increases while that of the other decreases With your dose of atropine you presumably produce a fairly specific inhibition of parasympathetic activity Do you have any observations of the effect of blocking just the sympathetic activity?

Levine Dibenamine in the rat gives protection against a formalin stress

Nickerson Do you have any dog experiments?

Levine No I do not

Nickerson From your data you have concluded that in the absence of the proper steroids, overactivity of the autonomic system can be deleterious I wonder if we cannot think about this problem in somewhat different terms Perhaps it is not so much the balance between steroids and autonomic activity as the fact that the animal lacking steroids is simply more sensitive than the normal to various deleterious factors As you pointed out very small amounts of norepinephrine kill adrenalectomized animals Our experiments indicate that norepinephrine will also kill normal animals but it takes a somewhat larger dose I suspect norepinephrine is a stress to both and that the adrenalectomized animal is simply more sensitive to it

Levine I should agree to that kind of interpretation except for some observations we made on the mesoappendix preparations In an adrenalectomized animal placed under sodium pentobarbital for the regular

observation of the mesoappendix preparation, the initial picture of the circulation in the mesoappendix was almost the same as in the normal animal, except, perhaps for less vasomotion As time went on, while the animal was observed with the mesoappendix exposed on the stage of the microscope deterioration occurred in the vascular bed.

Topically applied norepinephrine at the beginning of the experiment, showed the same order of activity as in the normal animal that is the vessels were quite sensitive Later there was loss of sensitivity to topically applied norepinephrine. Topically applied adrenocortical extract (containing hydrocortisone) will restore locally the sensitivity to norepinephrine without changing the situation in the animal as a whole Therefore, we feel that it is not just that the adrenalectomized animal is more sensitive because of something that happened somewhere else, but that actually the adrenal steroids are necessary for the direct reactivity of the vessels They seem to be necessary in a certain amount for many other things to act—the so-called permissive action of Ingle (19) Perhaps this is another instance of permissive action

Nickerson I would agree that the adrenal steroids are probably exerting a permissive action but this would not necessarily preclude looking at the normal and the adrenalectomized animals as only quantitatively different

Levine Yes I agree wholly

Fremont Smith Perhaps there are certain quantitative amounts of norepinephrine that are appropriate for the normal animal and when that amount has been used up any excess is deleterious It may be that the amount that can be used appropriately in the adrenalectomized animal is so slight that almost any norepinephrine is deleterious In other words if there is a combination of norepinephrine with tissues and a larger amount is possible in the normal animal than in an adrenalectomized animal, it would explain this situation and it would be acting differently because it became deleterious at a lower dose.

Levine I do not know We wanted to follow this up but we got off into a completely different program

Nickerson I should look at the problem this way When an animal or patient is on the verge of circulatory collapse any additional adrenergic stimulus whether it is sympathetic nerve activity or infused norepinephrine is deleterious, and the adrenalectomized animal is simply less capable of withstanding the deleterious effect.

Burton This really is a change of subject but I am very much stimulated by your thinking on these experiments I wonder if in your thinking you include the possibility that in our ordinary procedure for inducing shock *i.e* lowering the pressure, we are doing a physiologic

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adrenalectomy so that no steroids can come out of the adrenal cortex and this is the cause of irreversibility Is this a possibility? If so it suggests an experiment in which the blood supply of the adrenal cortex is kept normal while the rest of the animal is shocked, to see whether or not irreversible shock can be induced

Remington Now we must go back not 5 years, but 15 The suggestion had been made that shock might involve adrenal deficiency perhaps by exhaustion After a shipload of beef adrenals on its way from Argentina to Germany had been seized, it suddenly seemed important to those who were directing the war-time shock research to see if experimental proof for this notion could be found Considerable effort was made in various laboratories in which large amounts of adrenal hormones were given to animals in which shock had been produced by most if not all of the recognized conventional shock producing procedures, and the consensus was that there was no definite improvement in the circulation of the nonadrenalectomized animal

Frank We did direct assays of corticosteroids in adrenal venous blood samples in dogs before and during hemorrhagic shock

Levine There is more adrenal output?

Frank Not necessarily more, but it is extraordinary how well the adrenal can maintain its output of corticosteroids despite severe blood loss Reduction in adrenal blood flow must be extreme down to about 17 per cent of the normal blood flow before reduction in the steroid output can be counted on (20 21)

Fremont Smith Does adrenocortical blood flow follow renal blood flow?

Frank No it is independent As a matter of fact in some ways it is opposite, Dr Fremont Smith If the arterial pressure is raised with norepinephrine in hemorrhagic shock the *adrenal* blood flow will increase and the *renal* blood flow will drop

Levine Dr Burton, from our point of view and from clinical experience as well I should agree with Dr Remington s standpoint that it is more difficult to produce shock in the normal animal or individual than in the adrenalectomized subject Adrenal steroids have no effect in the normal but in the adrenalectomized animals the steroids restore to normal the resistance to shock inducing procedures In other words the adrenocortical block comes in as another one of the causes of the decrements of Dr Knisely

Burton I gather that the procedure where the blood pressure is dropped to 30 mm Hg for example does not result in absence of out flow

Fremont Smith I should like to make one comment on Goldblatt's work on renal hypertension (22) He could not produce renal hypertension in the absence of the adrenal cortex unless adrenal cortical hormone was supplied by injection This, of course, ties in also with the relationship which Dr Shorr and his group (23 24 25) have shown *i.e.*, the need for a functional adrenal cortex in order that the vasoexcitor substance which gives the epinephrine hypersensitivity may be produced Dr Zweifach and he showed that such hypersensitivity occurs in the compensatory stage of shock which we are trying to prolong, and have been prolonging by these experiments So it seems to me there is a tie-back into that original observation at this time and that the possibility that the same process which produces renal hypertension in the Goldblatt mechanism is the process which gives the compensation in the early stages of shock. It seems to me we are possibly tying a whole series of factors in together at this point.

Remington I should like to leave a strong question mark on the implication that adrenal crisis has the same cardiovascular basis as does the shock of which we have been speaking

Fremont Smith Adrenal crisis? You mean adrenalectomized shock?

Remington A hypotensive state that can be precipitated by but small amounts of what might be considered traumatic procedures be it the injection of a vasconstrictor drug a very small hemorrhage, a slight infection, or merely the administration of anesthesia

Levine Dr Remington would you agree that it is not qualitatively different?

Remington No I cannot agree with that It remains to be shown that the two states are qualitatively alike

Levine I should say that all the stresses which will lead to crisis in the adrenalectomized animal will, if prolonged and big enough lead to vascular collapse in the normal animal

Remington One major exception comes to mind Adrenal crisis can be produced by the giving of a relatively small plasma transfusion It can also be induced by the giving of a saline infusion

Haist Is cold one of these stresses?

Levine I used cold in the rat as a stress and cold stress was not protected by dibenamine

Nickerson In evaluating responses to cold stress it is important to keep in mind the fact that agents such as dibenamine and dibenzyline reduce cutaneous vasoconstriction and thus subject the treated animal to more stress than the control animal is subjected to at the same ambient temperature

Fremont Smith I should like to make just one more suggestion, that

a sulfhydryl mechanism of a ferritin type could operate not only in the liver but also possibly where norepinephrine acts on the vascular system, that is, on the neuromuscular junction or on the smooth muscle itself. It is conceivable that we are doing something that operates on these two mechanisms, *i.e.*, on the liver and on the vasomotor system simultaneously, using the same biochemical cellular metabolic process.

Nickerson: We do not have complete evidence yet, but, as a result of our studies on the reaction of dibenzyline and dibenamine with receptors, we have the impression that sulfhydryl is involved in the groupings (with which norepinephrine and epinephrine react to produce their excitatory effects).

Haist: How can one reconcile the fact Dr. Nickerson presents, namely, that the administration of norepinephrine can lead to shock, and the fact which has been demonstrated, that the administration of norepinephrine to the shocked animal can promote survival?

Nickerson: Has the latter been demonstrated?

Haist: Yes, we have found that in rats shocked by a clamping procedure the survival resulting from the continuous slow infusion of norepinephrine solution over a period of 24 hours was about as good as with dextran.

Nickerson: The interpretation of this result depends on the extent of fluid loss into the damaged tissue. The older literature contains one observation of significant protection against shock by a vasoconstrictor (26). Epinephrine in oil was administered to animals in which shock was produced by manipulation of the intestine. When the vasoconstrictor was given prior to the manipulation, much less engorgement and discoloration of the intestinal tract occurred. In other words, the constriction prevented the animal from being subjected to the same degree of stress as the controls. A similar factor of altered fluid loss into damaged tissue might be involved in the rats subjected to tourniquet shock.

Haist: It is a possibility. The limbs are swollen but we have not measured the degree of swelling in the norepinephrine infused animals. This procedure nevertheless has a very good effect on survival.

Levine: Dr. Haist, would you say that perhaps a certain amount of additional vasoconstriction during one stage in the shock picture would help the tissue circulation but that more than that would lead to tissue anoxia by vasoconstriction?

Haist: I must say that I find this particular result with norepinephrine infusion rather hard to reconcile with the effects of sympathetic blocking agents, but it is a real effect. Your suggestion may help in its explanation.

Remington The situation is really quite different Dr Nickerson, in producing shock with norepinephrine, is dealing with a prolonged hypertensive period The overly stimulated heart is forced to eject against the high arterial pressure through this period Finally something gives way Must we insist that only the peripheral arterioles were involved in this shock?

Shorr I think it could be cardiac failure rather than peripheral vascular collapse

Remington Certainly those working with the hypotensive state that follows a coronary infarct speak of shock affixing the adjective cardiogenic

Frank In dogs bled to hypotension, norepinephrine seems to change the distribution of blood flow Renal blood flow diminishes Saggital sinus flow coronary sinus flow and adrenal venous flow increase. Distribution of blood flow changes but in general not the cardiac output It is very hard to demonstrate that you are improving their survival. The animals become unresponsive to the drug and go on in shock and die

Dobson If in a burned animal blood pressure goes down and you try to maintain it with norepinephrine, you have to give more and more This is a situation where it is impossible to give enough norepinephrine

Fremont Smith It is an impossible situation from the start because the animal is in a hypotensive shock state He has no pressure You have nothing to work with

Knisely Following severe burns over the back and rump of grey hound dogs the blood passing through and away from the burned territory is heavily agglutinated Microscopic observations made in the lungs of such animals show that the masses of sludge are carried up to the lungs and trapped in the tips of pulmonary arterioles Probably the plugging of great numbers of such pulmonary arterioles decreases the flow of blood through the lungs and decreases the filling of the left heart (27 28,29)

Green Dr Nickerson do you know whether blocking drugs other than dibenzyline have sulfhydryl activity?

Nickerson As far as I know they do not The type of chemical reactivity responsible for the dibenzyline action on sulfhydryl compounds is not characteristic of the other agents However the possibility that they may have some effect on sulfhydryl groups cannot be ruled out without direct observations

Green If they do would it not be interesting to try them as dibenzyline has been tried in these procedures for inducing shock?

Baez. In this regard may I ask Dr Nickerson whether any homologue of dibenzyline which acts on the sulfhydryl enzymes, would necessarily block the receptors?

Nickerson No One of the intriguing things about the action of dibenzyline is its specificity Although the drug will react readily with a variety of sulfhydryl compounds *in vitro* the amount of dibenzyline necessary to produce essentially complete blockade of responses to epinephrine and norepinephrine is only enough to react with less than 0 1 per cent of the total body sulfhydryl On the other hand, compounds related to dibenzyline, but without the appropriate aromatic groups to produce adrenergic blocking activity react equally well with sulfhydryl in the test tube (8)

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HUMORAL FACTORS IN EXPERIMENTAL SHOCK

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I SHOULD LIKE TO DISCUSS some of our recent data which have led us to adopt certain reservations concerning the causal relationship between particular systemic humoral factors and the circulatory collapse which develops during hemorrhagic and traumatic shock

The use of a wide range of experimental models to study the shock syndrome has served to introduce a complexity of contributory factors with an uncertain relation to the basic derangements of this ubiquitous syndrome. Especially controversial are the mechanisms responsible for the deterioration of the peripheral circulation during protracted hypotension induced by hemorrhage Our own investigations were directed for the most part toward clarification of the role of blood-borne agents in the vascular sequelae during the progression of the so-called irreversible shock syndrome Observations on the changes in the capillary bed of the mesentery in the dog and rat led us to conclude that the shift from a compensatory to a decompensatory tendency was related either to the presence or absence of particular humoral substances affecting the response of the small blood vessels The pattern of behavior in the terminal vascular bed of the mesentery indicated the existence of two discrete phases subsequent to both trauma and hemorrhage. Initially there appeared a series of compensatory adjustments characterized by increased vasomotor activity heightened arteriolar sensitivity and selective restriction of blood flow to the most central and direct channels With protracted hypotension the compensatory behavior in the vascular bed was progressively replaced by a set of decompensatory stigmata as manifested by a failure to sustain the ischemic, restricted pattern of blood flow hyporeactivity of the muscular elements and increased venous stagnation, often to the point of complete stasis The prognosis for recovery by blood replacement measures was pro-

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gressively less favorable, the longer the decompensatory tendency was permitted to develop. Irreversibility was uniformly associated with a full quota of decompensatory activities.

Since the physiology of the small blood vessels has indicated that their distinction from the large vascular units lay in their high degree of responsiveness to humoral factors of both local tissue and systemic origin, the possible appearance of agents with *vasoactive potentialities* during the shock syndrome was therefore investigated in particular tissues and in the bloodstream. Biologically active agents with a "vasculo-toxic" action were sought for by using the terminal vascular bed of the rat mesentery as a test object. Concomitant with the characteristic vascular changes in the mesentery of the shocked animal, there appeared in the blood during the two principal phases of the syndrome certain principles which reproduced in the terminal vascular bed of the test rat the same sort of behavior which was apparent in the shocked host. The suggestion was then advanced that particular humoral substances of renal origin (VEM) might be causally related to the hyperreactivity seen initially and a hepatic factor (ferritin, VDM) to the subsequent hyporeactivity during the terminal phase of the reaction.

A good deal of criticism was leveled against the validity of extrapolating from observations in the mesentery to general systemic phenomena. The vascular derangements in the mesentery were obviously by themselves of no direct importance in the shock syndrome. It therefore became necessary to obtain comparable data in sites of greater significance in the over-all cardiovascular adjustment, particularly in tissues such as the liver and bowel, which show profound congestion and evidence of decompensatory trapping of blood during the latter phase of the shock reaction. Direct observations of blood flow through the liver are difficult to carry out continuously and are not sufficiently revealing to support or to disprove the importance of particular homeostatic factors. We therefore turned our attention to other visceral structures which could be more satisfactorily studied by the observational technique. The areas selected for microscopic study of the circulation were those in which such studies could be carried out without destroying the integrity of the vascular bed. As indicated in Table XXXVII, five structures were examined in the anesthetized rat. These tissues included the bowel, surface of the urinary bladder, and several mesenteric appendages other than the omentum or meso-appendix. In addition, skin and skeletal muscle were selected to represent two areas which appeared to show no decompensatory tendencies during hemorrhagic shock.

The evidence to be presented indicates the difficulty, perhaps even

TABLE XXXVII

Threshold Concentrations of Vasoconstrictor Agents Required to Bring About Closure of the Terminal Arterioles and Their Metarteriolar Extensions in Selected Vascular Beds

	Threshold Concentration*	
	Epinephrine ($\mu g/ml$)	L arterenol ($\mu g/ml$)
Skin (undersurface)	0.03	0.20
Urinary bladder	0.05	0.50
Mesorchium	0.50	2.0
Mesosalpinx	0.125	0.25
Mesoappendix	0.25	0.50
Skeletal muscle	0.001	0.0005

*Mean value for 10 rats for each tissue

the impossibility on the basis of our present knowledge, of relating the over all circulatory picture during shock to any single set of vaso-active principles

Burch Was that work done by capillarioscopy?

Zweifach It was all done by direct visual examination in other words, we are repeating the same thing using other tissues

Fine Was the urinary bladder studied by transillumination?

Zweifach The bladder is allowed to rest on a hollowed-out lucite support through which the light is transmitted

Burch Was the muscle done by reflected light?

Zweifach The technique of studying the spinotrapezius muscle in the rat has been published (1) The animal is placed on its side and the light is conducted under a flap of muscle through a lucite rod The lateral fascial aponeurosis of the muscle is drawn flat against the surface

of the lucite rod by several surgical sutures through this attachment so that the muscle itself is not handled directly

Burch It seems to me that the trauma might be rather extensive and might interfere with the innervation of the muscle preparation.

Zweifach There undoubtedly is some trauma involved in cutting through the skin and overlying connective tissue The muscle itself is not disturbed except to free one edge of its aponeurosis By keeping the muscle continuously irrigated with a Krebs-gelatin solution we hoped to minimize the abnormal situation created by its exteriorization. The innervation was left intact so far as we could ascertain This was the only skeletal muscle bed that could be prepared satisfactorily for microscopic study without drastically curtailing the reactivity and vasomotor behavior of its terminal vascular bed

Kinsely Are there bursae on this muscle?

Zweifach The spinotrapezius is directly under the skin so that very little connective tissue has to be removed to free its upper surface for study

In the experiments cited in Table XXXVII the response to topical epinephrine and norepinephrine(L arterenol) was determined in the selected vascular beds on the same set of vessels the terminal arterioles, and their metarteriolar extensions Vascular reactivity in the various tissues was compared on the basis of the threshold concentration of epinephrine or of arterenol required to bring about closure of these muscular units It is obvious that a considerable range of reactivity to topical vasoconstrictor agents exists In skeletal muscle the vessels were considerably more sensitive to norepinephrine than to epinephrine The latter agent in threshold doses produced a transient contraction followed by a prominent phase of dilation This situation makes it difficult to evaluate the precise significance of epinephrine potentiation or inhibition, as established by mesoappendix bio-assay relative to skeletal muscle circulation

Nickerson Do you have data on reactivity to epinephrine and arterenol administered intravascularly?

Zweifach A comparable gradient of reactivity values was found with intravenous epinephrine

Nickerson These differences are not a matter of drug penetration?

Zweifach The problem of penetration was checked by introducing the vasoconstrictor agents with a micropipet directly into the tissue adjacent to the vessel the same relative order of reactivity could be demonstrated in the tissues listed in Table XXXVII

Furchgott Did you find the same order of tissue reactivity for both epinephrine and arterenol?

Zweifach The closest approximation we have of the precise threshold doses was obtained in the microinjection studies Our microinjection data with L arterenol are much less complete than with epinephrine and I would rather not make a more definitive statement in that regard

The terms VEM and VDM have been used consistently in the past to indicate a vasoexcitor principle of renal origin and the vasodepressor ferritin. In order to avoid confusion we have introduced the symbols P and I P denotes potentiating substances and I inhibitory agents The test stimulus is then indicated as a subscript *i e* an epinephrine potentiator is designated as P and an arterenol potentiator as P_a

Test substances were administered intravenously to anesthetized rats exactly as in routine mesoappendix studies In Table XXXVIII beside the item, *skin* the response of the skin vessels is recorded, and beside the item *skeletal muscle* the response in the terminal vascular bed in muscle is reproduced, etc. None of the agents produced

TABLE XXXVIII

Displacement of Vascular Reactivity in Different Tissues by P_e and I Agents*

	Kidney VEM	Renin	L arterenol	Liver VDM	Ferritin	Histamine
Skin (undersurface)	o	P_e†	o	o	o	I_e†
Skeletal muscle	o	o	P	I_e	o	I_e
Mesoappendix	P	P_e	P	I	I_e	I_e
Urinary bladder	I_e	P	o	o	I	P
Mesorchium	P_e	P_e	o	I	I	I_e

*Administered in amount found to elicit positive reaction in mesoappendix
†P = epinephrine potentiation I = epinephrine inhibition

Reprinted, by permission, from Zweifach, B W., and Metz, D B Regional differences in response of terminal vascular bed to vasoactive agents. *Am J Physiol* 182, *155 (1955)*

the same sequence of potentiation or inhibition of the epinephrine response in the different tissues. Certain substances actually produced opposite effects, as in the case of histamine or of kidney VEM. It was apparent, because of the variable pattern of behavior in different regions, that no clear-cut conclusion could be drawn concerning the depressor or excitatory potentialities of blood borne substances to the circulation as a whole.

Selkurt: How was the kidney VEM prepared for such tests?

Zweifach: Kidney VEM was prepared from rat tissue slices incubated as for Warburg studies in an atmosphere of nitrogen for 30 to 45 minutes. The clarified supernatant was then injected into the circulation, about 0.25 ml. usually being sufficient to elicit a positive P response.

In Table XXXIX are indicated the results obtained with a single large specimen of blood (25.0 ml.) drawn from a hypertensive patient. Previous studies (2) have indicated that such bloods contain an admixture of vasoactive substances which in the rat test give a neutral test reaction. Aliquots of the same blood specimen were injected into different rats, each with a particular tissue exposed for observation. A neutral bio-assay was obtained in one tissue, a potentiated effect in

TABLE XXXIX

Hypertensive Blood Sample Assayed on Vascular Bed of Different Tissues

	Preparation	Bio-assay
Rat	Mesentery	Neutral
	Skin	P *
	Skeletal muscle	I *
Hamster	Mesentery	Neutral
	Cheek pouch	P

P = epinephrine potentiation, I = epinephrine inhibition.

another and an inhibitory reaction in a third. The implications of this experiment rest not with the precise reactions obtained but with the demonstration of the diversity of responses which the same vascular components exhibit in different tissues to the same biologic agents.

The following experiments (Table XL) bring out another important consideration basic to proper evaluation of humoral regulatory influences. In line with the hepatorenal concept the assumption has been made that vasoexcitor and vasodepressor materials were associated with a vascular tendency toward vasoconstriction and vasodilation respectively. Vasoactive agents were introduced systemically, either intra-arterially as shown here, or intravenously. The effect on arteriolar caliber in skeletal muscle was then compared with the shift in reactivity which accompanied the vasomotor change. Reactivity to topical L-arterenol is indicated as arterenol threshold concentration (A.T.C.) and to epinephrine as epinephrine threshold concentration (E.T.C.). It can

TABLE XL

Terminal Vascular Bed in Skeletal Muscle
Intra-Arterial Injections

Agent	Dose	Change in Caliber	Reactivity Change	
	(μg)		A.T.C.*	E.T.C.†
Epinephrine	1.0	vcs**	I_a††	Neutral
L-arterenol	1.0	vcs	P_a††	P_e††
Ferritin	0.001	vcs	P	Neutral
Mecholyl	0.03	vdil**	I_a	I ††
Histamine	3.0	vcs	I_a	P

A.T.C. = L-arterenol threshold concentration.
†E.T.C. = Epinephrine threshold concentration.
(both applied topically)
vcs = vasoconstrictor; vdil = vasodilator
††I = arterenol inhibition; P = arterenol potentiation; P = epinephrine potentiation
and I = epinephrine inhibition

be seen that in some instances vasoconstriction was accompanied by an inhibitory response to arterenol whereas no change in reactivity to epinephrine developed

Fremont Smith Do these threshold values represent intravenous or topical reactions?

Zweifach The vasoactive substance was introduced in this particular set of experiments via a catheter in retrograde fashion through the common carotid artery The material is then carried by the arterial circulation to the skeletal muscle The dose was adjusted to produce a visible change in arteriolar caliber The constrictor or dilator response usually persisted for 1 to 2 minutes Subsequent to this, after the vessels had resumed their original caliber the responsiveness of the vessels involved was determined by the conventional end point of a topical stimulus using either L arterenol or epinephrine and was recorded as noted above.

Burton Isn t the dissociation of epinephrine and arterenol reactions a peculiar phenomenon?

Zweifach Apparently in skeletal muscle the responses to arterenol and epinephrine did not shift concurrently (3) Actually epinephrine was found to inhibit the subsequent response to L arterenol

Burton Putting epinephrine in by the blood stream should not produce the same change as adding some norepinephrine topically

Zweifach I don t think that I have made my point clear Epinephrine produced vasoconstriction whether introduced via the blood stream or directly into the tissue However subsequent to the vasoconstrictor episode the smooth muscle elements of the arterioles were now less responsive to topical epinephrine than originally A similar predisposing effect was observed on the response to local norepinephrine following a vasoconstrictor episode induced by local epinephine

Fine Are these observations made in the same animal?

Zweifach The material listed in Table XL represents observations compiled from as many as 6 to 8 different animals and their average is indicated in each category

Burton You put the epinephrine in the blood stream and get the direct effect Do you wait until that is gone before you add the topical application or do you do both simultaneously? If the direct effect is gone then practically you would not expect it to have potentiated or inhibited

Zweifach The purpose of these experiments was to determine whether a constrictor or dilator tendency was necessarily always accompanied by a vasoexcitatory or vasoinhibitory behavior of the blood vessel We found that certain constrictor agents produced a visible

effect on vessel caliber which was then followed by a protracted period of vascular hyperreactivity to subsequent stimuli. We also found that other equally effective constrictor agents produced an effect which was followed by a period of hyporeactivity. A comparable situation existed for vasodilator factors, both L_e and P_e reactions appearing in such experiments.

Knisely: I have been studying the physics of the flow of blood in various systems. In a place where two rivers come together, such as the Missouri and Mississippi, the streams come together and run along beside each other in a common river bed but they do not mix for quite a distance. Contrariwise, in a system where a large artery feeds into smaller branches, an injection made into the main artery stream may be carried down into one of the side branches and most or all the injected material may go into one or another of the side branches. One or another of the other side branches may receive none of the injected material, or very little. Did you put anything into the material you injected, such as a colored dye, which would permit you to know that the material you injected was going past your microscope?

Zweifach: In these experiments, either a suspension of carbon or a solution of Water Blue was introduced into the arterial catheter in order to ascertain whether the injected substance reached the tissue under microscopic study. In the case cited, the material reached the muscle mass by way of the brachial artery. A total volume of 0.25 to 0.5 ml. was injected. The tissue was instantly blackened or blued when these tracers were introduced. We therefore know that the test material reached the target structure. Both the intra-arterial and the intravenous route were used to rule out possible secondary effects on other organs. The intra-arterial route made it possible to introduce vasoconstrictor doses of epinephrine or norepinephrine without affecting the pulmonary circulation. Comparable doses sufficient to produce vasoconstriction could not be readily administered intravenously.

Fine: Isn't it possible you may be getting different effects because of different local concentrations from the two routes of administration? With one unit dose intravenously you get vasoconstriction. If the concentration of the epinephrine applied locally is wholly different, you get a different local response.

Zweifach: In the data under discussion the material introduced into the blood stream served merely to elicit a vasoconstrictor or vasodilator reaction. We are not concerned here with the precise concentration required to introduce a given effect. The assumption is made that on the average the concentration reaching the target structure in the tissue under observation will be of about the same order of magnitude. The

subsequent degree of inhibition or potentiation of vascular reactivity was then titered more precisely by grading the response to a local test stimulus

Fremont Smith Contrary effects can be noted in different vascular beds That is to be expected When typhoid vaccine is given and there is complete vasoconstriction in the skin with a blood pressure of 60 mm. Hg for example, then 20 minutes later there is a complete vasodilation in the skin although the blood pressure is still 60 mm. Hg it is inconceivable that there has not been compensatory opposite reactivity of blood vessels under those circumstances The whole series of phenomena indicate that vascular beds in different parts of the body behave in opposite directions in many circumstances perhaps more often than not

Zweifach The experiments clearly indicate that vasoactive factors produce their effects by a variety of mechanisms even in a particular tissue and that broad extrapolation to other tissues is not indicated under any circumstances

Further evidence along this line is provided by the experiments summarized in Table XLI Representative constrictor or dilator agents

TABLE XLI

Terminal Vascular Bed in Skeletal Muscle Topical Application of Vasoactive Agents

Agent	Dose I V (µg/ml)	Change in Caliber	Reactivity Change
Epinephrine	0 001	vcs + vdil†	I **
L arterenol	0 0005	vcs	P **
Ferritin	0 0001	vcs	P
Histamine	0 01	vdil	P
Mecholyl	0 02	vdil	I **

Topical epinephrine or L arterenol
† [illegible] vasoconstrictor [illegible] vasodilator
‡ [illegible] inhibition P [illegible] epinephrine potentiation [illegible]
[illegible]

were applied onto the surface of the skeletal muscle preparation. Subsequent to the vasomotor episode, the reactivity status of the terminal arterioles was recorded by noting the response to either epinephrine or norepinephrine It can be seen that epinephrine had a dual effect in skeletal muscle. The terminal arterioles contracted shortly for a period of 10 to 20 seconds and then showed a pronounced dilator phase which persisted for periods of 7 to 10 minutes During this dilator period and for periods up to 45 to 60 minutes after the vessel has resumed its normal caliber the response to L arterenol was definitely suppressed as indicated by the I_s symbol in column 4 On the other hand a vasoconstrictor episode induced by intravenous L arterenol had a potentiating effect on the subsequent reaction to topical epinephrine Ferritin, interestingly enough, produced a clear-cut vasoconstrictor reaction in skeletal muscle when given intravenously Furthermore, the subsequent reactivity pattern was one of potentiation and not of vasoinhibition, as develops uniformly in the mesentery Histamine, in the concentrations employed produced a dramatic vasodilation and augmented flow through the terminal vascular bed in muscle Throughout the period of vasodilation, the vessels remained highly reactive to topical epinephrine Mecholyl on the other hand, induced a profound vasodilation together with a sustained vasoinhibitory reaction to topical epinephrine

Remington. When you say vasoconstriction, do you restrict the term to the arterioles or adjacent small arteries which is the most common usage, perhaps unfortunately, among physiologists?

Zweifach In preparations of this sort it is possible to study vessels ranging in size from small arteries about 500 μ in diameter to veins almost 1000 μ in diameter These are vessels which we place in the category of large vessels According to our definition the arterioles are those vessels which range from 40 to 60 μ in size, have a single layer of smooth muscle and are the first vessels of the arterial tree to give off capillary branches directly to the tissues It is obvious that a hard and fast line cannot be drawn between a small artery and its arteriolar extension There is no good evidence to indicate whether these two sets of vessels behave uniformly in the same fashion to particular stimuli or whether their responses differ

Remington If you are now speaking of an action of VEM and VDM on the arterioles themselves, this would seem a reversal of the statement made 5 years ago that their action was confined to vessels lying beyond the arterioles and that they would therefore have no necessary effect upon resistance as it might change with arteriolar size, which we presumably are measuring

Zweifach The agents listed in Table XLI produced a clearly evi

dent contraction of the arteriole and brought the blood flow in the capillary bed to a complete standstill

Green Do you find that the vessels in the muscle bed respond in the same manner to successively repeated doses of epinephrine?

Zweifach With repeated threshold doses of epinephrine, the vessels actually become refractory and vasoconstriction could no longer be elicited After an interval of 5 to 6 minutes threshold doses were again effective

Green In the dog epinephrine initially may induce vasodilation but with repeated doses the responses change to marked vasoconstriction

Furchgott You have indicated in Table XL that histamine induces vasoconstriction and in Table XLI that vasodilation appears

Zweifach There is a dissociation between the vasomotor effects of histamine depending upon the mode of administration When the drug was injected intra arterially constriction appeared in the vessels of the skeletal muscle preparation When applied topically vasodilation was observed

Furchgott By varying doses can you get the same response by the two procedures?

Zweifach No Irrespective of the dose the effect is the same with each mode of administration

Furchgott To what do you attribute the difference?

Zweifach The dilator effect of histamine administered topically may be an indirect one mediated by virture of its action on some tissue constituent

Furchgott In perfusion studies vasoconstriction was often observed with histamine But with better preparations in which peripheral tone was maintained by adding small amounts of epinephrine to the perfusion fluid or by using serum, dilation was uniformly obtained with histamine

Nickerson As I recall the work of Burn and Dale (1) one abnormal condition which readily converted the normal histamine vasodilation to constriction was hypoxia

Zweifach Histamine administration by way of the intra arterial route produced an immediate and abrupt vasoconstriction of the arterioles metarterioles and precapillary sphincters in skeletal muscle When histamine was applied topically over a wide range of concentrations these same vessels tended to dilate rather than to narrow

Green Is it possible to modify this response in any way by abolishing reflexes?

Zweifach We have not carried out any experiments along this particular line

Nickerson One other question on the ferritin You seem uniformly to get vasoconstriction in muscle Is this a pronounced effect?

Zweifach Yes the response is highly reproducible

Nickerson Can you rationalize this with the absence of a pressor response to ferritin?

Zweifach When ferritin is administered either intravenously or intra arterially no changes in blood pressure develop In this respect the term vasodepressor is a misnomer Presumably whatever decompensatory tendencies ferritin may exhibit operate chiefly in vascular beds other than muscle possibly in the splanchnic area

Nickerson Can you be sure that the responses you see are a result of effects on the vessels in your microscope field as distinct from possible effects upstream? The walls of the arterioles are under tension at all times and if an effect on a somewhat larger vessel feeding the arteriole causes the pressure to drop this will give the appearance of an active constriction, even though the effect is not on the vessel in your field of vision This is Dr Burton s critical closing pressure

Zweifach The largest arterial vessels visible on the surface of the skeletal muscle preparation are 500 to 750 μ in size Neither the caliber nor the rate of flow through these vessels was appreciably affected by the amounts of ferritin administered

Nickerson The systemic pressure need not change This type of effect has been invoked in attempts to explain the apparent dual effects constriction and dilation of histamine in perfused limbs I am not sure it can be fully substantiated but the idea is that the constrictor effect is exerted on somewhat larger vessels than the dilator effect. The apparent net effect depends on the state of the vascular bed In its normal resting state the very small vessels dominate the peripheral resistance and histamine produces a fall in resistance When these small vessels are dilated the next larger vessels contribute a larger component to the resistance and their constriction gives an apparently qualitatively different response

Knisely Consider the hydraulic events when one is making an injection slowly into a large artery having rapid flow It should be possible and it should often happen that the injected material would pass down the axial stream and have no contact or minimal contact with the internal surfaces of the walls of the artery until the injected material reaches the narrower vessels where the small branches come off There is no reason why the intramural smooth musculature of the large stem artery should be penetrated by the material injected Differences in the volume injected and the rates of injection would determine the width of

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the stream of injected material passing down the artery and thereby determine the size of the artery with which the injected material first comes in contact

Burton I am certainly impressed with the difficulty of deciding whether what you see in small vessels is active or passive because pressures change as a result of an active change somewhere else This would not apply to topical application

Zweifach The observed constriction was sudden and complete without any prior slowing of blood flow in the feeding arteries or arterioles Admittedly changes in caliber can develop secondarily to changes either proximal or distal to the terminal vascular bed Those effects however are usually presaged by marked alterations in blood flow A further consideration is the fact that in these experiments extremely small concentrations of vasoactive materials were used The smaller blood vessels are more sensitive to these dose levels than are the larger blood vessels

Burton If the perfusion pressure of a frog's mesenteric bed is lowered enough the most extraordinary things happen Some vessels narrow and others suddenly become wider because of these passive changes

Knisely Do you think those are because of passive changes or because of differences in nutrition (5)?

Burton The nutrition of the mesentery is not a very potent factor

Knisely Why don't you think so?

Burton Because the mesentery stays reactive for hours

Knisely The same reactive changes can occur with no changes in perfusion pressure at all

Burton This really is not a point at the moment The fact that so often a flow can be seen to reverse completely through this part of the bed impresses me with the difficulty of deciding that what you saw was caused by some active reaction at that place rather than something that was just off the field

Nickerson The thing that is bothering a number of us is the apparently active histamine induced constriction of the arterioles

Zweifach I can only report what the rat did and I think I am reporting it correctly

Nickerson It is not a question of what you saw The arterioles undoubtedly decreased in caliber but your observation might be correlated more readily with peripheral resistance and blood flow measurements if we might assume that the action of histamine in producing this response was not entirely on the arterioles in your field of vision

Frank Is it feasible to test some of the points by microinjection just proximal to the site of observation?

Zweifach I think perhaps it would defeat your purpose.

One other feature serves to emphasize the difficulty of establishing the vasomotor potentialities of blood borne agents Figure 33 is a

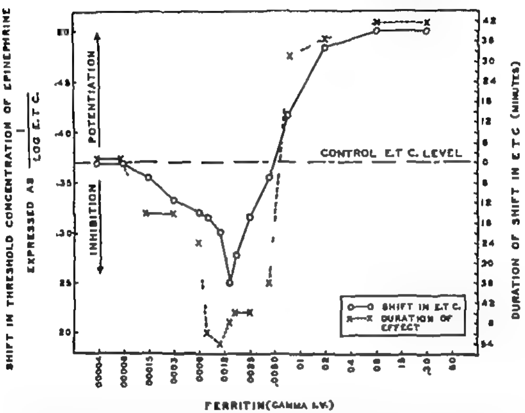

FIGURE 33 Shift in character of vasoactive response with change in concentration of ferritin administered intravenously The range (0 00015 to 0 005 μg ferritin N/mL content)* over which ferritin could be classified as an I_e substance is extremely narrow Beyond this range, only P_e effects were obtained. Comparable prozone phenomena were indicated on basis both of the shift in E.T.C. (solid line) and the duration of period during which vascular reactivity was displaced (broken line)

curve indicating the displacement of vascular reactivity which appeared in the rat mesentery when different concentrations of ferritin were injected intravenously Each point on the curve represents the maximum displacement of the epinephrine threshold as averaged from four to six animals A total of seventy five rats was studied, only one test being

* Since ferritin is not a pure compound, but a tissue extract, its activity cannot be expressed in terms of weight. Being a protein, the nitrogen content (N) represents the most accurate frame of reference.

conducted in each animal. At the extreme end of the dilution curve, no vasoactive changes were detected. As the concentration of ferritin increased the E.T.C. was progressively depressed. Reactivity values were determined as the precise amount of epinephrine required to elicit a standard constriction, not merely the presence or absence of a constrictor response. With concentrations of ferritin above 0.002 *to* 0.005 μg./ml. (N content) the inhibition of the epinephrine response became less pronounced. Neutral reactions were frequently observed in this concentration range. Beyond 0.01 μg. ferritin injections potentiated the epinephrine response to a moderate degree. No I effect was observed with amounts of ferritin in excess of 0.01 μg., up to 5 μg. A comparable biphasic pattern was obtained with various ferritin doses in another mesenteric appendage, the mesorchium of the male rat.

Nickerson: It refers to concentration effects, a situation in which too much as well as too little of one component of a reaction system reduces the response.

Zweifach: This phenomenon is not unique to ferritin. A similar effect on vascular reactivity was observed with different doses of histamine or pitressin injected intravenously (Figure 34). At low concentrations these drugs seemed to compete with epinephrine and to inhibit the local vasoconstrictor response. With higher doses a typical potentiating action was elicited.

Remington: Do you think the appearance of vasoconstriction is related to the size of the vessel being studied?

Zweifach: All of the reactivity values were determined on the metarterioles and precapillaries of the terminal vascular bed.

Shorr: Were these effects also manifested on the larger blood vessels?

Zweifach: The hyperreactivity which developed with ferritin could also be observed in the collecting venules.

Shorr: Was there much spread between the values?

Zweifach: A certain degree of individual variability was encountered but never to the extent that I values went above or P values below the control base line. The greatest variability was encountered at the two extremes of the reactivity curve.

Srikantia: Can you passively transfer this potentiation effect into another normal test rat? I ask this because we know that when ferritin is injected into a rat its vasodepressor effect can be transferred into another rat by infusing the blood of the first rat into the second. Similarly, is this potentiation effect also transferable?

Zweifach: I am not [illegible] if a single dose of ferritin is injected into a [illegible] oved from that rat and infused in [illegible] another [illegible] oduced

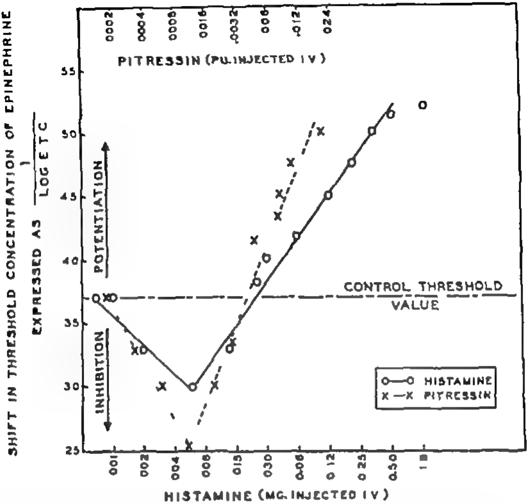

FIGURE 34 The appearance of both I and P vasoactivity with changes in the amount of pitressin and histamine. I_a effects appear with the lowest effective doses and P reactions in the higher range of concentrations Pitressin is expressed as p u., pressor units, and histamine as mg (milligrams)

Srikantia The point I wanted to make is this With injections of high concentrations of ferritin, is there the formation of some substance that causes a potentiation of the effect of topically applied epinephrine?

Zweifach I imagine that such a possibility exists The injection experiments of the type presented *do not distinguish* between direct and possible indirect effects of ferritin on vascular behavior However it should be pointed out that a comparable situation was encountered with injection of histamine or pitressin as indicated in Figure 34

The observation that shifts in vascular reactivity coincide with the appearance of appropriate vasoactive principles in the blood stream during shock represents a major argument favoring the hepatorenal concept A set of experiments was conducted to ascertain whether it would be

conducted in each animal At the extreme end of the dilution curve no vasoactive changes were detected As the concentration of ferritin increased, the E.T.C. was progressively depressed Reactivity values were determined as the precise amount of epinephrine required to elicit a standard constriction, not merely the presence or absence of a constrictor response. With concentrations of ferritin above 0 002 to 0 005 μg /ml (N content) the inhibition of the epinephrine response became less pronounced. Neutral reactions were frequently observed in this concentration range Beyond 0 01 μg., ferritin injections potentiated the epinephrine response to a moderate degree No L effect was observed with amounts of ferritin in excess of 0 01 μg up to 5 μg A comparable biphasic pattern was obtained with various ferritin doses in another mesenteric appendage, the mesorchium of the male rat

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Remington Do you think the appearance of vasoconstriction is related to the size of the vessel being studied?

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Shorr Were these effects also manifested on the larger blood vessels?

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Shorr Was there much spread between the values?

Zweifach A certain degree of individual variability was encountered but never to the extent that L values went above or P values below the control base line The greatest variability was encountered at the two extremes of the reactivity curve.

Srikantia Can you passively transfer this potentiation effect into another normal test rat? I ask this because we know that when ferritin is injected into a rat its vasodepressor effect can be transferred into another rat by infusing the blood of the first rat into the second Similarly is this potentiation effect also transferable?

Zweifach I am not sure that if a single dose of ferritin is injected into a test rat and blood is then removed from that rat and infused into another rat the VDM effect will be produced

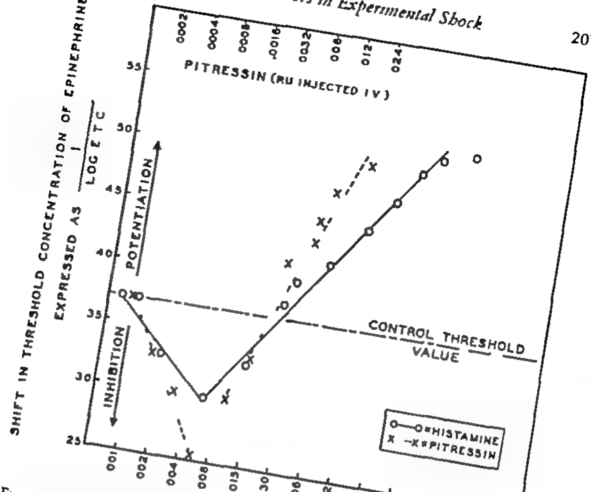

FIGURE 34 The appearance of both I_e and P vasoactivity with changes in the amount of pitressin and histamine. I_e effects appear with the lowest effective doses and P reactions in the higher range of concentrations. Pitressin is expressed as p u., pressor units, and histamine as mg (milligrams)

Srikantia The point I wanted to make is this With injections of high concentrations of ferritin, is there the formation of some substance that causes a potentiation of the effect of topically applied epinephrine?

Zweifach I imagine that such a possibility exists The injection experiments of the type presented do not distinguish between direct and possible indirect effects of ferritin on vascular behavior However it should be pointed out that a comparable situation was encountered with injection of histamine or pitressin as indicated in Figure 34

The observation that shifts in vascular reactivity coincide with the appearance of appropriate vasoactive principles in the blood stream during shock represents a major argument favoring the hepatorenal concept A set of experiments was conducted to ascertain whether it would be

possible to alter the vascular phenomena during shock in a predictable direction by these vasoactive agents The accompanying protocol (Figure 35) indicates vascular reactivity in the mesentery systemic blood pressure and the concomitant bio-assay values The rat was bled an amount which was not fatal but which was sufficient to produce a typical pattern of compensatory readjustments The *mesenteric circulation* showed hyperreactivity *as manifest by spontaneous vasomotion and by the* enhanced response to topical L arterenol or epinephrine. During this period blood removed for bio-assay elicited a P response At this stage a continuous infusion of small amounts of vasoactive material was instituted either intravenously or intra arterially using a motor driven micropump In this instance, horse-spleen ferritin was administered. In addition to the dose range employed other experiments were carried out in which the material was injected during shock over a considerable dose range. This particular dose is used for illustration since blood removed at the points where arrows are shown on the graph,

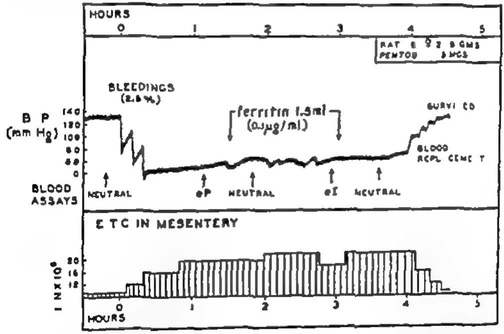

FIGURE 35 Intravenous administration of ferritin during initial stage of shock when blood bio-assays were P in character and mesenteric vessels showed a hyper reactive vasomotor pattern. *Despite infusion of* VDM *(ferritin)* continuously for almost 2 hours, mesenteric vessels continued to show a P response coincident with a shift to I titer in blood. (The symbols eP and eI in this figure correspond to the symbols P and I as used in the text and in this legend) Reprinted, by permission, from Zweifach, B. W., and Metz, D B Relation of blood borne agents acting on mesenteric vascular bed to general circulatory reactions. *J Clin Investigation* 34 653 (1955)

was found to have sufficient titers of ferritin to elicit a positive I. assay in a test rat Despite this circumstance, absence of a significant effect on vascular reactivity will be noted. The hyperreactive state, apparent prior to the infusion was sustained throughout the period of ferritin administration. The infusion was terminated after 120 minutes blood replacement was carried out, and the neck wounds were closed This rat survived the procedure.

Burch Does the stage of estrus make any difference in the reaction of the test rat?

Zweifach The experiments were done on both male and female rats The degree of variability was no greater in the female rat, so as to make it doubtful that estrus affected these reactions

I should like to present further evidence to show that on the basis of our present information we cannot causally relate the two sets of phenomena, namely vascular changes and blood borne principles during shock

In Figure 36 a comparable experiment is shown in which the animal

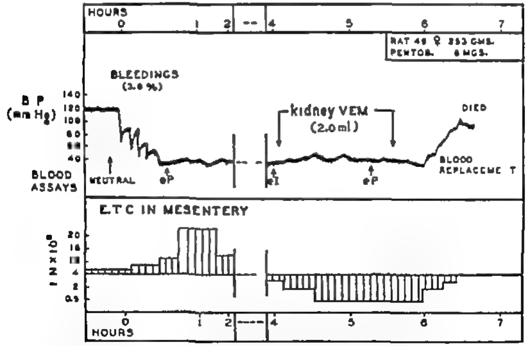

FIGURE 36 Intravenous infusion of vasoexcitor material (VEM) following 2 hours of drastic hypotension at stage where blood specimens contained I material and mesenteric vessels were hyporeactive. No influence on vascular behavior despite shift of blood bio-assay from I to P during period of infusion (90 min.) (See legend for Figure 35 re eP and eI) Reprinted, by permission, from Zweifach, B W., and Metz, D B Relation of blood borne agents acting on mesenteric vascular bed to general circulatory reactions *J Clin Investigation* 34 *653 (1955)*

was kept at hypotensive levels until a clear-cut decompensatory tendency had developed Blood samples were found to contain an inhibitory principle by passive transfer to test rats The circulation in the mesentery showed the onset of the inhibitory phase of vascular reactivity At this stage a continuous infusion was instituted with a preparation of kidney VEM (one part kidney to three parts Kreb s solution) The vasoexcitor material had been prepared by the conventional technique of anaerobic exposure. As little as 0.2 ml of the supernatant fluid produced in a test rat a positive P effect The kidney VEM was injected at a sufficient rate with the micropump to achieve a P titer in the bloodstream. Despite the appearance of P activity in the blood the mesentery continued to show a vasoinhibitory pattern of behavior No changes in blood pressure could be detected The animal died despite blood replacement

Shorr Dr Furchgott, you worked with VEM produced from kidneys. What can you tell us about its activity?

Furchgott It was difficult to work with as we could not purify it. We would concentrate it and then overnight the activity would decrease, considerably

Zweifach I think the point to be emphasized is that with the infusion of this kidney mixture in our experiments a blood level was reached which on rat assay gave a positive P test

Furchgott There may be a dilution effect here which complicates the situation You find that either the vasoexcitor or vasodepressor effect is dominant as far as the mesentery of the rat in hypotension is concerned But when blood from the rat in hypotension is injected into the test rat, a dilution occurs The response of the mesentery in the test rat may be different from that of the experimental rat because of the dilution.

Zweifach This type of argument would hold for any bio-assay procedure on blood specimens Certainly the titer of VEM was high enough to counteract the VDM present in the blood prior to the infusion It was anticipated that the preponderance of P activity in the bloodstream should in some ways alter the depressed vascular reactivity in the mesentery

Furchgott I think that a dilution effect might be important If it is assumed that there are two principles involved dilution might affect the activity of one more than the other

Zweifach Would you say that the test rat was reacting differently to this admixture of VDM and VEM than did the shocked rat?

Furchgott What I mean is this If the dose response curves of two oppositely acting agents show very different slopes then there is the

possibility that a more concentrated mixture containing a fixed ratio of the agents might give a qualitatively different response than a more diluted mixture containing the same ratio

Zweifach I think the protocol illustrated in Figure 36 proves the point which I had in mind in the design of the experiment An attempt was made to mimic the humoral situation which develops during shock. Kidney VEM was selected for this purpose since it apparently appears in the blood stream during the course of the shock reaction. The dose of VEM administered was sufficient to shift the blood titer of vaso-activity from an I reaction to a P test reaction. The dilution factor does not enter into consideration here since the dose range was arrived at arbitrarily by injecting an amount sufficient to produce the desired effect.

Nickerson Particularly with mixtures it is important to know some thing about the slopes of the dose response curves of the components if the effects of dilution on the net response are to be interpreted adequately

Zweifach Table XLII is another example selected from our recent publications which indicates the complex relationship between the appearance of vasoactive material in the blood stream and a particular set of vascular phenomena.

Patients were selected at random in the outpatient clinic of a local hospital, with the understanding that they had not had breakfast and were not receiving medication. Blood samples were sent to us in code Each of the specimens was tested over a series of dilution ranges by the conventional bio-assay test and vasoactivity compared under vari ous categories of clinical diagnosis

When normal healthy controls were studied positive L or P activity by the rat mesoappendix test was rarely encountered The bloods were taken from healthy students residents, and co-workers in the laboratory On the other hand in the case of people who came to the clinic either because they were sick or felt they were sick we invariably found the presence of P or I activity in the blood Neutral bloods were routinely fractionated and in the clinic group of patients the blood was found to contain a mixture of both L and P material The findings in this series could not be related to the cardiovascular status of the patient. The evidence would appear to indicate that in man these substances enter the circulation of people who are apparently ill, whether the illness is psychosomatic or somatic in etiology

Engel We have also found the same sort of relationship in carbohy drate metabolism between normal healthy medical students and people who were in the hospital either because of somatic or functional

TABLE XLII

Bio assay of Bloods in Random Sampling of 90 Clinic Patients

Diagnosis	Age (yr)	Blood Pressure (mm Hg)		No. of samples with Specific Vasoactivity		
		Systolic	Diastolic	P **	I **	Neutral
Arthritis	50 to 73	110 to 160	80 to 90	1	3	2
Asthma	40 to 70	110 to 140	80 to 90	—	2	—
A.S.H.D.*	60 to 70	140 to 150	70 to 80	5	2	1
Diabetes	42 to 72	140 to 170	80 to 100	2	1	1
Gastrointestinal disorders	25 to 50	120 to 140	70 to 90	5	4	3
Hypertension	30 to 72	165 to 210	90 to 115	6	12	2
Hyperthyroid	30 to 50	120 to 140	70 to 80	2	2	1
Ca breast or gastrointestinal tract	40 to 66	105 to 120	60 to 70	—	5	1
Obesity	29 to 59	100 to 158	70 to 85	1	3	—
Rheumatic heart disease	20 to 47	90 to 130	70 to 80	1	4	1
Assorted normotensives†	27 to 64	90 to 125	60 to 85	7	5	5
Per cent in each category				33	48	19
Healthy controls	23 to 42	100 to 140	70 to 82	2	1	18

A.S.H.D. = *Arteriosclerotic heart disease*

†Includes patients with epilepsy, bronchitis, nontoxic goiter, poliomyelitis, psychosomatic complaints, syphilis, and vasomotor rhinitis

**P = epinephrine potentiation and *I* = epinephrine inhibition

Reprinted by permission, from Zweifach, B. W., and Metz, D. B.: Relation of blood-borne agents acting on mesenteric vascular bed to general circulatory reactions. *J. Clin. Investigation 34: 653 (1955)*

disease (6) We tested their response to cortisone in carbohydrate metabolism People who said they were sick always showed abnormal response, whereas the healthy ones showed a normal response. What it means, I do not know

Shorr A great deal of the interesting material Dr Zweifach described above relates to the specific humoral concept of shock which has been advanced by our laboratory as a working hypothesis I first heard some of this work presented a few years ago and since then we have investigated some of it in our laboratory The fact that we have continued to explore the implications of this working concept should indicate to you that I remained unconvinced that it is possible to exclude participation of these humoral factors by the evidence submitted at that time and I am taking the same position now

The publications which Dr Zweifach did not have time to report in full detail, included, amongst other aspects a consideration of the deficiencies of the rat mesoappendix test (7) I think that an impression was created by these reports which is not justified i.e., that the quantitative deficiencies of the test are such as to cast doubt on the experimental findings which our laboratory has reported, with respect to the alterations in the VEM and VDM systems in the course of shock. I do not think that Dr Zweifach meant to imply that in his article

Zweifach Our recent publications have clearly pointed out the deficiencies of the mesoappendix method particularly with respect to quantitation. It is unfortunate that the bio-assay data have been expressed as numbers an implication of quantitation is read into the data whether intentional or not. Comparisons have been made i.e., between bio-assay values in one instance of 24 minutes or a sixfold change in reactivity with another bio-assay which showed a twofold change, etc. The evidence unmistakably indicates that this is not possible There is no doubt that the mesoappendix method represents a valid qualitative measure of vasoactivity However with respect to specificity and to quantitation, there is a considerable body of evidence to indicate that extrapolation in this regard has no sound basis

Shorr Have you obtained evidence that the sequence of events relating to the humoral factors in shock needs to be modified?

Zweifach The evidence indicating a particular temporal sequence of humoral and vascular changes in the mesentery during shock is in my opinion unequivocal and our recent findings (8) do not discredit the original data in this regard

Shorr We then have it clearly stated that the implications of your investigations of the validity of the rat mesoappendix test in no way alter the findings which we have published here or elsewhere

On the other hand we have not been able to confirm a number of your experiments whose implications were that the continued presence of ferritin in the circulation results in hyperreactivity

Burton What do you think the standard error in such experiments might be?

Zweifach There is a considerable scatter Thus a given sample will yield assay values varying from 20 to 36 minutes in duration and from a twofold to sixfold change in intensity

Shorr *I do not think that you appreciate the fact that despite its* limitations this is a useful bio-assay method

Baez Figure 37 was plotted from data obtained in our laboratory by

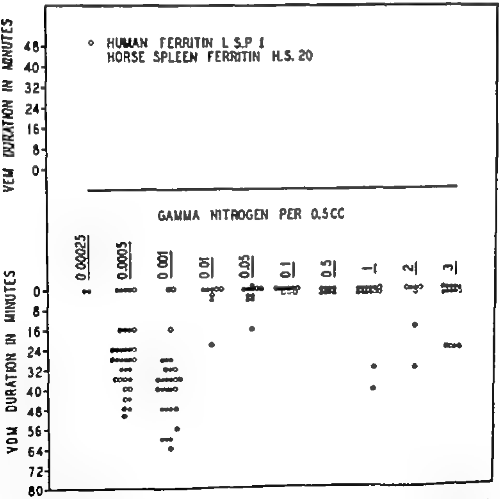

FIGURE 37 Duration of hyporeactive response to single I V injection of crystalline ferritin in amounts ranging from 0.0005 to 3 µg N Fresh rat used for each bio-assay

six different workers over the past year. Each dot represents a single bio-assay. The material used was crystalline ferritin prepared by Dr Mazur and Dr Livingstone in our laboratory from horse spleen or human liver, spleen, and placenta. Vasoexcitor responses are shown above and vasodepressor responses below the horizontal line dividing the figure. Maximal activity with both ferritin preparations was obtained at amounts ranging from 0.0005 to 0.001 μg ferritin nitrogen. With increasing concentrations up to 3 μg ferritin nitrogen the great majority of the assays were neutral and we failed to observe the hyperreactive response in the mesoappendix which Dr Zweifach found with amounts of 0.01 to 0.3 μg ferritin-nitrogen. Two black dots are shown above the horizontal dividing line indicating hyper reactive responses. When these occurred, our stock supply of ferritin was examined by Dr Mazur and found to have deteriorated. It was therefore discarded. This leads us to believe that denaturation of the ferritin sample may account at least in part for the discrepancy in the findings of the two laboratories.

Zweifach. Do you mean that you arbitrarily discard a given batch of ferritin because it does not provide the desired I_e effect?

Baez. Yes, because it was found to be denatured. That is, it could not be redissolved, whereas it was originally soluble.

Zweifach. The data which you present clearly corroborate the existence of two sets of vasoactivity with ferritin at high and low doses respectively. The majority of the values fall at the neutral range when amounts of ferritin over and above 0.01 μg are administered.

Baez. I am not denying that the majority of the tests became neutral with higher doses. I am merely showing that no hyperreactivity whatsoever occurred even with doses many times greater than those you injected.

Zweifach. What you are disputing then is the development of hyper reactivity as the dose is increased. The possibility has been raised that P activity is associated only with particular batches of ferritin—chemically altered in some way. I must admit that this possibility exists but I think the point to be emphasized is that ferritin in different concentrations produces a variable effect ranging from an inhibitory reaction to no reaction.

I should like to point out, however, that the particular ferritin preparation used in our studies gave clearly evident reproducible and inhibitory reactions at concentrations of 0.001 to 0.0075 μg ferritin N/ml, a range of activity almost identical with that of your own data.

Shorr. The side reactions were probably caused by the denaturation

of the sample The prozone phenomenon is something we have known about for a long time This effect was first observed in our laboratory and reported in 1951 (9) When we worked with anaerobic spleen and found no VDM activity although we knew it was rich in ferritin we tried diluting the samples and then we found the expected vasodepressor activity Our usual proportions are 1 gm of tissue to 5 ml of Ringer phosphate solution but with spleen samples we had to dilute ten times to a 1 to 50 instead of a 1 to 5 ratio Since that time all assays which appear to be neutral are repeated at one or more dilutions We do not fully understand the reason as yet but higher concentrations of ferritin often give neutral rat tests Our greatest activity occurs with 0 0005 to 0 001 μg ferritin N

Zweifach Your maximum range of activity falls between 0 01 to 0 005 μg of ferritin Presumably your argument then is that this is the level achieved in the blood during shock Accordingly this level is never surpassed because otherwise a prozone would be encountered a finding never achieved even in the most profound state of shock

Shorr Yes

Zweifach Blood samples obtained during shock with a vasodepressor activity (presumably ferritin since the effect can be counteracted with antiferritin serum) can be diluted from threefold to fivefold without losing inhibitory properties of these specimens In the data presented in your graph with only a small change from 0 005 to 0 001 μg ferritin N/ml vasoactivity was lost

Shorr I do not disagree with you that the activity in blood or tissue extracts can be detected at several dilutions But my point is that although higher amounts of crystalline ferritin do give neutral rat test responses which are consistently vasodepressor only on dilution to my mind this is not related to the hyperreactivity at higher dosage levels which you obtained with rat or horse ferritin

Burton What is the mean duration of the VDM activity?

Shorr In Figure 38 at the 0 0005 μg level 30 out of 35 tests gave VDM activity ranging from 16 to 52 minutes in duration with the mean at 31 minutes the standard deviation was $\pm$ 9.8 and the standard error $\pm$ 1 8 five tests were neutral At the 0 001μg level 33 out of 35 tests were vasodepressor with duration ranging from 17 to 72 minutes with a mean of 43 minutes a standard deviation of $\pm$ 12 and a standard error of $\pm$ 2 1 two tests were neutral We have known for a long time that doubling the concentration does not double the duration In addition the response has at least one other characteristic intensity but we recorded only duration since attempts to measure the intensity may lead to hyperreactivity of the vessels Although the bio-assay has these

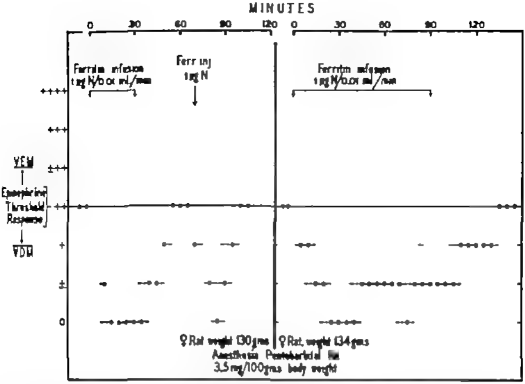

FIGURE 38 On the left, typical hyporeactive response obtained on intravenous infusion of ferritin at the rate of 1 μg ferritin N per 0 01 ml. per min. for 30 min. On the right (experiment suggested by discussion at this conference) infusion of ferritin at the same rate but continued for 90 min There was no change in the type of response.

distinct quantitative limitations amounts of ferritin can be detected by it which are much less than can be determined by chemical means

Pillemer Have you tested the effect of injecting human ferritin into rats?

Shorr It seems to make no difference which is injected into the test rat human, horse or dog ferritin.

Pillemer Doesn t it matter even though ferritin is antigenic?

Shorr Yes but with a single injection there is not time to build up antibodies

Pillemer Couldn t this prozone effect be caused by this?

Shorr I think not.

Pillemer Do you find the same type prozone if you inject rat ferritin into the rat? Has this been done?

Shorr We have not succeeded although we have not given it too much attention in crystallizing rat ferritin. Was your ferritin really pure and free from side effects? I believe you described the field affected as a broader one than the terminal vascular bed

Zweifach Such a widespread effect was obtained with continuous infusions of ferritin for periods of from 50 to 60 minutes or longer

Shorr Frequently that sort of response is seen with respiratory difficulty or with impure samples of ferritin

Baez. The effects we obtained by epinephrine applications to the mesoappendix during a 30-minute continuous intravenous infusion of ferritin at the rate of 1 μg N/0 01 ml /minute in an anesthetized rat are shown on the left side of Figure 38

Shorr These are gradations in refractoriness

Fine Would you explain to us what you have done? Is this injected into the rat and are you testing that rat?

Baez The test rat was anesthetized with 3 5 mg of sodium pentobarbital / 100 gm body weight and the mesoappendix carefully exposed for bio-assay (10) of vasotropic substances by direct microscopic observation of the minute muscular vessels The threshold reactivity to epinephrine was determined the ferritin infusion into the tail vein begun, and the epinephrine applications repeated at approximately 5 minute intervals

Fine What has pentobarbital to do with the effect on the mesoappendix?

Baez It is always used as anesthesia to hold the rat just quiet enough for continuous observation of the same field

Zweifach In attempting to compare the data which you present with those in our own experiments it is evident that your infusions were comparatively short, for 30 minutes whereas the experiments cited in Figures 33 and 34 represent infusions of approximately 2 hours duration

Shorr When does the shift in reactivity occur following the administration of ferritin?

Zweifach Between 55 and 75 minutes

Baez On the left side of Figure 38 there are two curves representing gradations of refractoriness At the first epinephrine application after the start of the ferritin infusion the sensitivity of the muscular vessels had declined slightly 10 minutes after this the reaction to the threshold concentration had completely disappeared and it did not return to control levels until about 40 minutes later that is 20 minutes after completion of the infusion At this time after checking the calibration a single injecion of 1 μg of ferritin was followed by a similar but less prolonged VDM effect This second VDM response is perhaps an indication that the return of the vascular sensitivity to threshold levels following the continuous infusion of 30 μg ferritin was not caused by

refractory receptors but rather by an effective rapid inactivation of the infused ferritin.

Green Are these normal animals?

Shorr Yes Dr Zweifach didn t show that experiment today He has published a figure (8) in which 45 minutes after the infusion had begun, there was a shift from hypo- to hyperreactivity with persistence of hyperreactivity until the infusion ended

Zweifach The point to be emphasized is that the 45 minutes to which you refer is an observation made 15 minutes after the cessation of infusion

Shorr That is true but also the period of the induction of hyper reactivity should have occurred within the framework of this experi ment

Zweifach Three separate batches of ferritin were used in our work Each of these preparations on dilution gave a positive rat test in the proper dose range Each aliquot was tested for biologic activity in a control animal before infusion.

Shorr The reason this point seems important to me is that these ex periments showing hyperreactivity on the continuous infusion, and on injection of higher doses of ferritin, imply that the status of the animal in shock, similarly exposed to its own ferritin, is one in which we should expect hyperreactivity

Zweifach The data presented in our recent publications do not ecessarily imply that the continuous presence of VDM should lead hyperreactivity They do however imply that at high concentrations ferritin no vasoinhibitory effects can be demonstrated What this phenomenon signifies relative to the shock syndrome remains to be shown.

Shorr As Dr Baez has said we regard these discrepancies between the two laboratories as probably having been caused by alterations from biologically sound ferritin preparations and we are convinced the hyperreactivity is an artifact

Zweifach Have you repeated experiments of the type reported here with your own particular ferritin preparation and shown a fall in blood pressure a countereffect on vascular reactivity during shock or found any significant differences from our own set of observations other than the disputed point regarding hyperreactivity with high doses of ferritin?

Shorr No

Haist Dr Shorr I am not quite clear You infused here continu ously for 30 minutes?

Baez The longest infusion was for 42 minutes

Haist I thought that Dr Zweifach showed the effect at 45 minutes

Shorr Yes but at 45 minutes the effects are still depressor though gradually coming back to neutral

Haist Since the effect does not appear until after 45 minutes is this a comparable experiment?

Shorr Do you consider 45 minutes the critical period for this effect?

Zweifach The important consideration here is the short period of ferritin administration in the study of Dr Shorr and Dr Baez. What remains to be done is to perfuse for longer periods as in our own work and see whether vasoactivity remains depressed Should your data with your own ferritin indicate hyporeactivity throughout it will be necessary to assume that our preparation of ferritin was in some way altered

Shorr We had regarded the large dose as the important factor but since you consider that the length of the infusion also may influence the results we will repeat the experiment using your conditions and put the result in the record As we said in the introduction to the first conference of this group, Repeating experiments exactly is made possible by conferences like this " (11)

Baez One difficulty with a 2 hour continuous infusion of ferritin is that the test rat will start to come out of the anesthesia and will need further injections of pentobarbital *

Shorr It is a long period In the experiment shown in Figure 38 with a 30 minute infusion there was enough ferritin activity in the blood so that hyporeactivity lasted 50 minutes Then at this time it wore off and the administration of another dose brought about the return of hyporeactivity

Haist It might be expected that there would be a greater accumulation of the ferritin with the longer infusion than with the shorter one

Shorr It depends on the amount You see what is happening is that the liver is removing the ferritin We found in our antidiuretic experiments that in contrast to the rather large amount going in intravenously the amount remaining in the blood was very small

Haist The ferritin concentration goes down gradually rather than abruptly

Shorr It always takes a little time for the hyporeactivity to develop

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Subsequent to this discussion, in similar experiments the period of continuous intravenous infusion of ferritin was lengthened to 90 minutes The results remained unchanged A typical experiment is shown on the right side of Figure 39 No hyperreactive responses were obtained in the mesoappendix vessels The depression of the vascular sensitivity to topical epinephrine was maintained for 45 minutes after the completion of the continuous infusion

Haist Is this related to an increase in the concentration of ferritin in the blood over a period of time?

Shorr No because in the mesoappendix test, as we regularly use it for bio-assay purposes we inject 0 5 ml. within about 30 seconds so that the whole concentration is there maximally at the first moment, and yet it takes time for the response to develop We sometimes see it after 3 minutes, at the first application of epinephrine, but it may take as long as 12 minutes to appear

Barton Dr Shorr I do not understand how you get those points in Figure 38

Baez. The values on the lower left hand side of Figure 38 merely represent the change in responsiveness as compared to threshold values The dots which fall at the zero level indicate complete unresponsiveness to the application of the epinephrine

Zweifach I might add that in our own experiments we felt it would be more useful to determine the precise concentration of epinephrine required to elicit a threshold response The graphs were then drawn plotting the reciprocal of the log of the concentration against time

Baez. Yes there is a difference in the method of testing with topical epinephrine, and I should like to point out that we purposely use the same threshold concentration of epinephrine throughout the test period Or to put it differently when unresponsiveness of the vessels develops following the injection of ferritin, we do not use stronger concentrations of epinephrine to elicit a response because we want to avoid possible hypersensitization of the target structure

Green. Do these points indicate the degree of response to a constant dose?

Baez Yes

Green Do they represent responses in a single animal and not an average of a series of animals?

Baez Yes

Shorr We have also not understood some of the data on VEM infusions Our kidney VEM preparations were not sufficiently concentrated for us to anticipate that on infusion they could neutralize the vasodepressor activity of blood in an animal in shock.

Zweifach The VEM was injected at a rate determined by the amount which served to maintain positive P activity in the blood

Shorr I should think that the VEM activity would be so dilute that when so slowly infused it could not help but be ineffective I am surprised you were able to detect it in the blood

Pillemer The terms *good* ferritin and *bad* ferritin are confusing to the chemist What do these terms mean? What are the criteria that

Haist I thought that Dr Zweifach showed the effect at 45 minutes

Shorr Yes but at 45 minutes the effects are still depressor though gradually coming back to neutral

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Shorr It depends on the amount You see what is happening is that the liver is removing the ferritin We found in our antidiuretic experiments that in contrast to the rather large amount going in intravenously the amount remaining in the blood was very small

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Burton Dr Shorr I do not understand how you get those points in Figure 38

Baez The values on the lower left-hand side of Figure 38 merely represent the change in responsiveness as compared to threshold values The dots which fall at the zero level indicate complete unresponsiveness to the application of the epinephrine

Zweifach I might add that in our own experiments we felt it would be more useful to determine the precise concentration of epinephrine required to elicit a threshold response The graphs were then drawn plotting the reciprocal of the log of the concentration against time

Baez Yes there is a difference in the method of testing with topical epinephrine and I should like to point out that we purposely use the same threshold concentration of epinephrine throughout the test period Or to put it differently when unresponsiveness of the vessels develops following the injection of ferritin we do not use stronger concentrations of epinephrine to elicit a response because we want to avoid possible hypersensitization of the target structure

Green Do these points indicate the degree of response to a constant dose?

Baez Yes

Green Do they represent responses in a single animal and not an average of a series of animals?

Baez. Yes

Shorr We have also not understood some of the data on VEM infusions Our kidney VEM preparations were not sufficiently concentrated for us to anticipate that on infusion they could neutralize the vasodepressor activity of blood in an animal in shock

Zweifach The VEM was injected at a rate determined by the amount which served to maintain positive P activity in the blood

Shorr I should think that the VEM activity would be so dilute that when so slowly infused it could not help but be ineffective I am surprised you were able to detect it in the blood

Pillemer The terms *good* ferritin and *bad* ferritin are confusing to the chemist What do these terms mean? What are the criteria that

you have established for something being good or bad? Is the ferritin that he is using good and is yours bad, or is yours bad and his good?

Shorr The criteria are those generally accepted for protein purity electrophoretic homogeneity and solubility at the isoelectric point The protein must be in the native state and not denatured

Pillemer Yes but there are various kinds of denaturation

Shorr That is true.

Knisely I wonder what specific physical and chemical characteristics can be used as criteria or as specific terms for the materials which are being used Is this a completely soluble small molecule material or is it a large molecule?

Shorr The molecular weight is 465 000 for apoferritin, the protein without the iron

Knisely I imagine that after this material is in the blood stream various different substances may become attached to it

Shorr What happens to it in the blood stream is something we can only speculate about at present

Knisely Thus the molecule with the complex of substances attached to it which arrives in different places in the body may not be at all identical to the molecule injected

Fremont Smith This may be true of anything injected anywhere at any time It is a general statement I think you can wonder for instance, about sodium chloride, what has happened to it and whether it is exactly as it was when it was injected We do not really know what happens to anything when it is injected into the blood stream

Nickerson Do you have available some of the criteria regarding solubilities etc?

Shorr The electrophoretic data have been published (12 13) I cannot agree that because certain substances have different actions on different parts of the vascular bed they therefore can have no physiologic significance

Zweifach The point at issue here is whether it is possible to predict from effects recorded in one area such as the mesentery the vascular behavior in other tissues The existence of both qualitative and quantitative differences in particular areas makes such generalizations extremely hazardous The inference has been made with regard to ferritin that the appearance of this agent in the bloodstream should either uniformly depress the circulation or exert such an overwhelming effect in one area that it would counterbalance the changes in other areas

Knisely It has been known for a long time that epinephrine for instance opens the hepatic outlet valves which is opposite to the

usual beginning medical school teaching that epinephrine causes arteries to contract

Shorr Yes We are all accustomed to thinking of biologic agents or endogenous factors, which have different actions on different parts of the vascular bed Their potential significance for the shock syndrome and their particular action in areas such as the splanchnic bed, which are demonstrated to be vitally affected in the syndrome, remain the object of continuing study

Zweifach Our data do not rule out a possible vasoinhibitory action of ferritin on the circulation in either the liver or the bowel. The highly specialized character of the liver circulation would make any direct inference from the mesenteric circulation *per se* highly speculative.

Shorr So that if the really critical change of events is taking place in the splanchnic bed then its action or lack of action elsewhere cannot be considered as ruling out the importance of this vasodepressor factor in shock

Zweifach Examination of the response to selected vasoactive agents administered either systemically or locally revealed an unpredictable pattern in five representative vascular beds These distinctions led us to conclude that blood-borne factors of this type probably do not serve a systemic function but rather represent local vasoactive factors at their site of origin This contention was further supported by the failure to influence vascular reactivity during either the compensatory or decompensatory phases of hemorrhagic shock by the infusion of the oppositely acting vasoactive substances

The necessity also exists to demonstrate that the decompensatory tendency as measured by the vascular response to epinephrine or to arterenol represents the basic lesion during the development of irreversibility It was not possible to substantiate the consistent association of vascular hyperreactivity with an increased responsiveness to other stimuli and the converse situation hyporeactivity with a diminished response to other stimuli The fact that identical changes in vascular reactivity could be instituted by purely local phenomena raises the question whether we are not dealing in the case of shock with regional reactions each brought into play by different sets of metabolic and neurogenic factors The development of decompensation may not necessarily be the consequence of a single set of humoral factors

Shorr: This postulates that VDM like substances may be produced locally

Zweifach: Equivalent potentiating and inhibitory effects were pro-

duced by local ischemia or by locally administered agents in every tissue which we have studied

Shorr Ferritin is widely spread throughout the tissues of the body and it is of great interest to us and possibly of importance for this concept, that these phenomena do not occur until the metabolism of the liver is converted to the anaerobic type with the release of ferritin

Engel Wouldn t a critical point be whether ferritin can induce these changes in the liver? In other words as I see the argument if it is said that the primary site of action is on the splanchnic bed then for ferritin to be of primary importance it must initiate the change in the splanchnic bed which in turn induces the anaerobic state in the liver Unless it can be induced in this way it becomes a secondary phenomenon and falls in the same category as the failure of inactivation of ferritin itself Ferritin falls in the same category as many other metabolic phenomena of the liver We have so much difficulty deciding whether these are important or incidental phenomena

Shorr Yes I agree

Zweifach Changes in vascular reactivity have been observed in particular segments of the capillary bed in the skin mesentery etc There is no comparable evidence dealing with the reactivity pattern relative to specific structural elements in the small blood vessels of the liver proper In fact the evidence indicates that the architecture in the liver is highly specialized and differs from that in other tissues We therefore find ourselves in a quandary to explain what influence ferritin would have on the hepatic circulation even if the material did exert an I influence on the muscular vessels

Engel The metabolic phenomenon must be initiated in the liver by ferritin affecting the splanchnic bed It would not make any difference what ferritin did to the liver itself though

Zweifach If the decompensatory tendency during protracted shock is to be associated with ferritin, and the major sites of blood sequestration are in the splanchnic viscera we must be able to relate the two sets of phenomena causally

Green I should like to ask in what other bed besides the mesoappendix ferritin has been shown to have this depressor effect?

Shorr I refer you to Dr Zweifach

Green We have studied the effects of ferritin in skeletal muscle but we never published the data because we were afraid the ferritin we had was not good We studied a wide range of doses just as we have in the studies of the adrenergic blocking drugs in skeletal muscle (1 1 15 16) but we could not demonstrate any blockade of epinephrine constriction

Baez. Were those studies performed in a normal dog preparation or in an animal that had already been shocked to a certain degree?

Green The ferritin is injected arterially, into normal dogs in the same way in which we injected adrenergic blocking drugs

Shorr We have never claimed or demonstrated any effect of ferritin on anything but the terminal vascular bed

Green If the VDM is supposed to have a traumatic effect and to impair the circulatory responses, shouldn t you be able to demonstrate this effect in some other bed than the mesenteric?

Shorr Not necessarily if the splanchnic area happens to be the crucial one.

Fine The whole circulation is depressed there is depression of flow everywhere Why shouldn t you find this?

Shorr That is true If we are correct in our assumption which is, after all, only a working hypothesis, that the crucial factor in the failure of recovery of the animal lies in the splanchnic area in particular in the set of events initiated in the liver then it would be necessary that this vasodepressor effect be exerted only in that area

Fine Why do you make that assumption?

Shorr I believe the whole evolution of the shock theory, not just our own work, points in that direction.

Burton Wouldn t the basis of the experiment, to support the claim that this factor or that is the important one, be shock? It seems to me we all have the right to be interested in these particular factors One would pick out dozens of them Dr Fine expressed a bacterial one, you think of VDM To me as an outsider the test of whether some thing is important in the shock syndrome is to see whether that partic ular agent when given to an animal, produces any change in the out come of the shock experiments

Fremont Smith It has got to be given at the right spot.

Shorr This would seem a very reasonable request however I should like to show a little later some evidence which demonstrates how danger ous it is to assume that if you want to test this hypothesis you can just inject some ferritin

Burton We have been using the criterion on other theories For instance some have said I give an antibiotic and it doesn t affect the shock picture. Shouldn t we apply the same criterion as to whether this is really important in shock?

Shorr Yes but these things are far more complicated than they seem on the surface and sometimes we rule out things and later on we find we had better rule them in again!

Dobson I want to say a word in regard to the importance of the

splanchnic area in shock as illustrated by changes in its circulation following burns. Here the liver circulation is depressed but less depressed than the rest of the circulation. The average liver circulation is down let us say to about 40 per cent whereas the cardiac output is down to 30 per cent of normal and sometimes much more than this (17) This means that the circulation elsewhere is down more than the cardiac output

Furthermore, there is some suggestion that later on in the post burn course, the liver circulation may increase Our method for measuring liver circulation does not work very well later on because phagocytic function is depressed (18) and we cannot accurately distinguish between phagocytic efficiency and blood flow changes But it may be that we are overemphasizing a depression of splanchnic blood flow

Knisely In 1912 Krogh showed that in human beings the liver and portal vein bed constitute a blood reservoir He also showed that the blood flow out of the hepatic veins is a major factor determining cardiac output. There are three papers in which he shows this namely Measurements of the Blood Flow Through the Lungs of Man (19), On the Influence of the Venous Supply upon the Output of the Heart (20), and The Regulation of the Supply of Blood to the Right Heart with a Description of a New Circulation Model (21)

The experiments were done upon Krogh himself and his colleague Lindhard In the analyses and discussions it is shown that (*a*) the heart cannot pump any blood which does not get to it (*b*) under ordinary conditions it pumps all the blood that it does get (*c*) the venous pressure at the point where the superior and inferior vena cava enter the heart is very low (*d*) extremely small increases and decreases of flow into the heart determine moment to moment changes in cardiac output and (*e*) the small decreases and increases of flow *from the* hepatic veins into the inferior vena cava determine the totals of the flow into the right atrium The significant point is that whenever the liver and portal vein bed begin to store blood the amount stored, even if continually moving is withdrawn from the general circulation and immediately thereby acts to decrease the flow in the superior and inferior vena cava The essential point for this argument is that very small changes *in the flow through the hepatic veins are determinative* in the mathematical sense of cardiac output under these particular conditions (22)

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PRESERVATION OF THE HEPATIC FERRITIN SYSTEMS AND PROTECTION AGAINST SHOCK BY *N* - (2-chloroethyl) - *N* - (cyclohexylmethyl) - ethylamine hydrochloride (G-D 131)*

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I SHOULD LIKE TO PRESENT some of our more recent studies (1) on preservation of the hepatic ferritin systems and protection against shock by *N* (2-chloroethyl) *N* (cyclohexylmethyl)-ethylamine hydrochloride (G D 131) These studies will illustrate how much the contact with the men and the give and take in these conferences has helped our work. About 3 years ago when we found that dibenzyline specifically affected the liver in shock, protecting the ferritin inactivating systems and preventing ferritin release (i.e preventing anaerobic production of vaso-active sulfhydryl ferrous-ferritin) we should have liked to consider that this provided some support for our theory about the evolution of the shock syndrome, but the interpretation of the results was clouded by the fact that there were, in addition to the above effects vascular changes brought about by the adrenergic blocking properties of dibenzyline

At about this time we talked with Dr Nickerson, who told us of the very interesting study he had done with Dr Gump (2) on the relation of the structure of adrenergic blocking agents to their action. Amongst the compounds examined in the effort to pinpoint the chemical groups necessary for this effect were some that had been slightly altered so that they had lost all adrenergic blocking action. Later, Dr Nickerson

*Aided by research grants from the National Institutes of Health, U S Public Health Service (Grant H 79) the Josiah Macy Jr Foundation the Research and Development Division, Office of the Surgeon General, Department of the Army (Contract No DA-49-007 MD-388), the Armour Laboratories and the Postley Fund. The skilled assistance of Iris Forbes and Anne Carleton in carrying out these studies is gratefully acknowledged.
†Deceased.

also studied a group of these compounds with Dr Harvey (3) showing that some of these altered compounds lacking adrenergic blocking properties still had like dibenzyline, the capacity to bind sulfhydryl groups Would you comment briefly Dr Nickerson?

Nickerson The structure-activity studies revealed that *several interesting* and somewhat complex factors are involved in producing the characteristic action of this type of blocking agent (2) However the basic factors of importance in this discussion are that saturation of the aromatic portion of the molecule provides agents which lack adrenergic blocking activity but do react with sulfhydryl whereas elimination of the alkylhalide moiety abolishes both properties

Shorr Dr Nickerson sent us a sample of one of these compounds in which the adrenergic property was abolished by the saturation of the benzene ring* This was G D 131 [*N* (2-chloroethyl) *N* (cyclohexylmethyl)-ethylamine hydrochloride] It was quite nontoxic in fact it had no discernible pharmacologic properties at the doses used We set out to explore it using drum shock in rats because that is the easiest form of shock to study It was absolutely without any benefit so we put it aside 3 years ago

At last it occurred to us that a trial of G D 131 in hemorrhagic shock might be worth while since aureomycin protected rats against hemorrhagic shock despite its meager benefit (at 60 minutes pretreatment) in drum shock The results were very interesting and led us to explore the possibility that without having any complicating vascular effects this agent might resemble dibenzyline in its effects on the ferritin regulating systems Such was indeed the case

Figure 39 shows the *in vitro* results with G-D 131 Ten micrograms in 5 ml were incubated with one gram of liver slices for 10 minutes

Nickerson Quantitative comparison of these blocking agents may become important to this discussion and I should like to point out that very low concentrations are effective in producing adrenergic blockade *in vitro* Our standard blocking concentration of dibenzyline is 0 05 μg /ml

Shorr The procedure is the same as Dr Baez described for *in vitro* studies with aureomycin or dibenzyline The liver slices were exposed to the agent for 10 minutes and then were washed after which they were incubated for 90 minutes in nitrogen As shown in Figure 39 no ferritin was released When the same anaerobic liver slices were then reincubated aerobically with ferritin they inactivated it as well as a

*Dr William Gump Givaudan Delawanna Corporation, also kindly sent us some of this compound

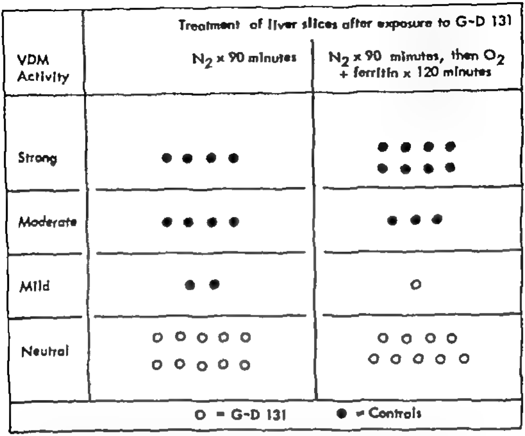

FIGURE 39 *In vitro* effect of G D 131 on the hepatic ferritin systems. Liver slices (1 gm.) incubated for 10 minutes at 37 5°C. with G D 131 (10 μg) in 5 ml. Ringer PO_4 medium in oxygen. Slices washed twice and reincubated as above Reprinted, by permission, from Baez, S., Srikantia, S G., and Shorr E. Influence on the hepatic ferritin systems of a tertiary amine, G-D 131 with beneficial effects in shock. *Proc Soc Exper Biol & Med 92, 61 (1956)*

normal liver does whereas the control anaerobic liver slices were unable to inactivate the added ferritin

Our data on G D 131 compared with dibenzyline in hemorrhagic shock in rats are given in Table XLIII The difference between the protection with dibenzyline and with G D 131 is not significant The uptake of blood was somewhat less in the treated groups than in the controls Here, then is an agent which regardless of what other unknown effects it may have is not an adrenergic blocking agent and which in concentrations of 80 μg/100 gm of body weight produces a high degree of protection against hemorrhagic shock

Figure 40 shows the bleeding pattern in these rats The period of maximal blood loss before uptake, is longer and ends later in both treated groups than in the controls

TABLE XLIII

Comparison of the Effect of Dibenzyline and G-D 131 on the Outcome of Hemorrhagic Shock in the Rat*

	Controls 0 2 ml saline	Dibenzyline 20 μg/100 gm body wt	G D 131 80 μg/100 gm body wt
Number of animals	22	19	22
Maximum blood lost† (Per cent body weight)			
Average	4 5	4 5	4 1
Range	3 5 to 5 3	3 3 to 5 5	3 5 to 5 0
Uptake of blood** (Per cent body weight)			
Average	1 0	0 5	0 5
Range	0 0 to 3 6	0 1 to 1 0	0 0 to 1.8
Per cent survival at 24 hours	27	63	77

Both agents injected into the tail vein 60 minutes before bleeding

†Total blood loss required to bring blood pressure to 30 mm Hg level

*Amount of blood spontaneously taken up from reservoir to maintain blood pressure at 30 mm. Hg for 2 hours

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You may recall a protocol of an experiment presented by Dr Baez at a previous conference (4) showing changes induced by dibenzyline in the behavior of the terminal vascular bed of the mesoappendix of the rat There was initial depression of epinephrine reactivity which was replaced as hemorrhagic hypotension developed by hyperreactivity In each category—vasomotion vasoconstriction of the arterioles and capillary blood flow—compensatory reactions persisted in the treated rat for much longer periods than in the control

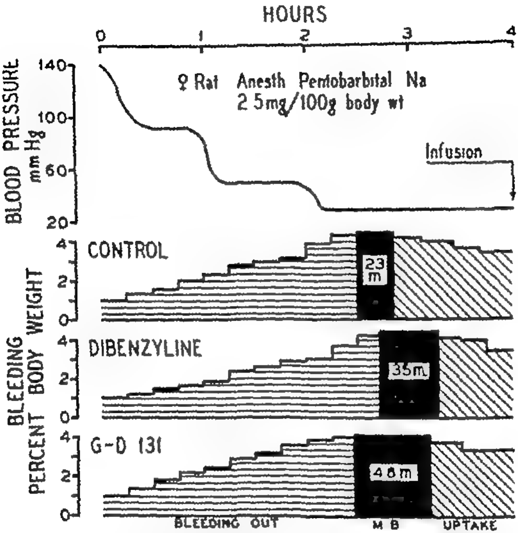

FIGURE 40 *Diagrammatic representation of the bleeding pattern in G-D 131 and dibenzyline-treated rats and their controls.*
MB = average maximal blood loss
m = average duration in minutes of the maximal blood loss

Figure 41 is a similar protocol with G-D 131 instead of dibenzyline In doses of 80 to 200 µg /100 gm body weight G-D 131 had no discernible effect on the terminal vascular bed of the normal rat However during the hemorrhagic experiment, compensatory types of vascular behavior persisted in the mesenteric bed of the treated rat as compared to the marked deterioration seen in the control animal

We asked ourselves why we got no benefit in drum shock when the results in hemorrhagic shock were so excellent Our experience with aureomycin in drum shock which Dr Baez has described previously

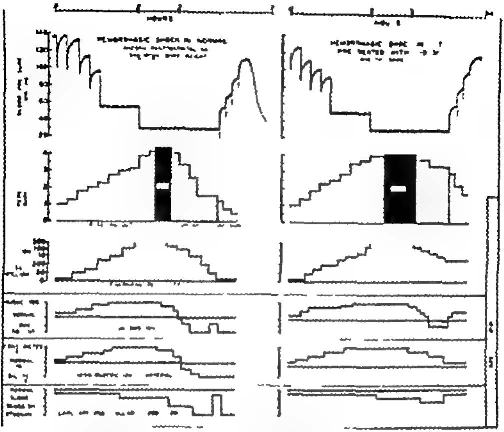

FIGURE 41 Diagram of peripheral vascular responses to standardized hemorrhagic shock in a normal rat and in one treated with G-D 131 80 μg./100 gm. body weight I V 1 hour prior to start of bleeding

aureomycin an hour before drum trauma we got very little protection but if we waited 2 hours and then drummed the rats, the protection was substantial

Fine That is providing you gave fluid too?

Shorr No extra fluid The controls were given the same volume of saline as was used to dissolve the drug for injection in the treated rats. They got only 0 2 ml

Fine You have about 77 per cent recovery yet you didn't give any fluid?

Shorr No fluid therapy was given. We looked at our hemorrhagic experiments and realized that injecting an agent an hour before the bleeding is begun leaves an interval of 3 hours before the liver becomes really hypoxic with the lowering of the blood pressure to the 30 mm. Hg level So we tried giving G D 131 *3 hours before the drum trauma* In Table XLIV the effect on the survival rate is shown 50 per cent

TABLE XLIV

Effect of Intravenous Pretreatment with G-D 131 On the Outcome of Drum Trauma in the Rat

	Saline	200 μg G D 131/100 gm body wt	
Time of injection	−60 to −180 minutes	−60 minutes	−180 minutes
Number of rats	94	28	93
Number of rotations	715	715	715
Number surviving at 24 hours	41	15	78
Per cent survival at 24 hours	44	53	84

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survived when 0.2 ml. saline was given 60 to 180 minutes before drumming The survival was the same when the G D 131 was given 1 hour before trauma but when it was given 180 minutes before, the beneficial effect was dramatic 84 per cent survived.

We have removed the livers from control rats and from those pretreated with G-D 131 at the end of our standard hemorrhagic shock procedure (Figure 42) and have found the ferritin mechanisms intact in the treated rats in contrast to the deterioration of these systems in the control rat livers Blood assays agreed, in general with the findings in the liver In other words, G D 131 exerts its protection of the hepatic ferritin systems *in vivo* as well as *in vitro*

Engel Doesn t this experiment in a sense answer the question I raised earlier? If I understand it correctly and with all its implications here is a situation in which you say that the liver does become hypoxic, am I right?

Shorr Yes in the controls

		Blood 0 5 cc	Liver slice incubation O$_2$ × 60 minutes	Liver slice incubation Plus ferritin in O$_2$ × 120 minutes
V E M	Strong			
	Mod			
	Mild	○		
	Neutral	○ ○ ○ ○ ● ●	○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○ ○
V D M	Mild	●	●	●
	Mod	○ ○	○ ● ●	●
	Strong	● ● ● ●	● ● ● ●	● ● ● ● ●

○ = G D 131   ● = Controls

FIGURE 42 Effect of pretreatment with G D 131 (80 μg /100 gm. body weight given into the tail vein 60 minutes before the hemorrhagic procedure) on hepatorenal vasoactive factors in blood and liver after 4 hours of hemorrhagic hypotension at or above 70 mm. Hg for the first hour at 50 mm. Hg for the second hour and at 30 mm. Hg for the following 2 hours. Reprinted, by permission, from Baez, S. Srikantia, S. G and Shorr E. Influence on the hepatic ferritin systems of a tertiary amine, G D 131 with beneficial effects in shock. *Proc Soc Exper Biol & Med* 92, 61 (1956)

Engel But it still succeeded in inactivating ferritin did it not?

Shorr We do not know whether the liver in the treated rat gets as hypoxic as the control liver

Engel Then, you do not really know where the agent is acting?

Shorr We know that in the presence of the agent the harmful effects of hypoxia are prevented

Engel Then what you are measuring is one parameter of the protection of the hypoxic liver

Shorr That is right

because it does not have anything to do primarily with the liver becoming hypoxic. Therefore, how can one distinguish between the abnormality in the liver with respect to inactivation of ferritin from any other metabolic phenomenon? What makes it any more important?

Shorr I should like to restate what you have said in this fashion The animal is exposed to a degree of shock which ordinarily affects the liver profoundly and which in particular damages the ferritin systems Regardless of what may happen to the over all oxidative metabolism of this organ, when we add an agent which preserves the ferritin systems, the administration of that agent and its effect on these systems are associated with survival. This doesn t mean that X, Y or Z may not be the core of the problem instead of ferritin But so far we have no better evidence or specific indication of what goes wrong

Engel Is there any way you can more or less specifically poison the ferritin inactivating mechanism in the animals protected with one of these drugs without knocking out a lot of other enzyme systems?

Fremont Smith Without hypoxia

Engel Hypoxia, presumably now *is not important. Apparently the* liver is working all right.

Baez. Since the blood flow is better and the over all dynamics of the splanchnic capillary bed are unchanged in the pretreated animals I wonder whether hypoxia develops in the liver to the same degree as in the controls

Engel It seems to me that the protective agents act by cutting down on the decompensating effect which would have resulted had ferritin been released In this case the critical experiment would be to devise some way of poisoning the particular mechanism for ferritin inactivation and showing that now the animal decompensates

Shorr We haven t done that. This agent is nontoxic in the doses we give. The LD_{50} is about 75 mg /kg or 7½ mg /100 gm., and the effective dose is 80 μg /100 gm. or perhaps even less we have not tried lower doses

Recovery is associated with the administration of an agent which has no vascular effects that we can discern, which is not adrenergic blocking, is not anticholinergic, nor antibiotic, in fact has no pharmacologic effects in the animal as far as we know but which has a very definite effect on the ferritin systems in the liver The inference that we draw is that the persistence of the compensatory phenomena under these circumstances is a result of the liver s being prevented from releasing something which is deleterious to these compensatory reactions

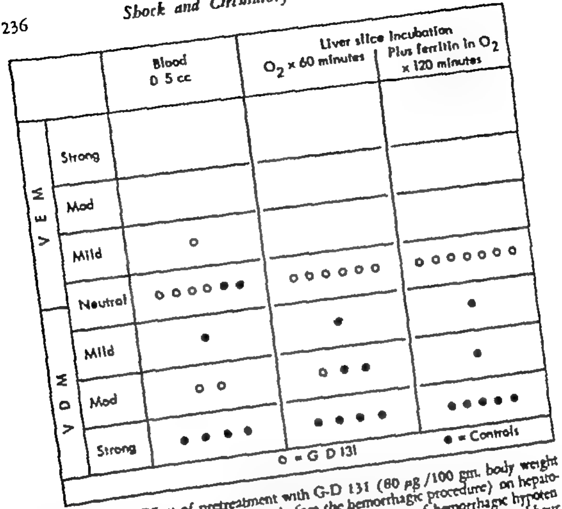

FIGURE 42 Effect of pretreatment with G-D 131 (80 μg/100 gm. body weight given into the tail vein 60 minutes before the hemorrhagic procedure) on hepato-renal vasoactive factors in blood and liver after 4 hours of hemorrhagic hypotension at or above 70 mm. Hg for the first hour at 50 mm. Hg for the second hour and at 30 mm. Hg for the following 2 hours. Reprinted, by permission, from Baez, S. Srikantia, S. G., and Shorr E. Influence on the hepatic ferritin systems of a tertiary amine G D 131 with beneficial effects in shock *Proc Soc Exper Biol & Med* 92, 61 (1956)

Engel But it still succeeded in inactivating ferritin, did it not?

Shorr We do not know whether the liver in the treated rat gets as hypoxic as the control liver

Engel Then, you do not really know where the agent is acting?

Shorr We know that in the presence of the agent the harmful effects of hypoxia are prevented

Engel Then what you are measuring is one parameter of the protection of the hypoxic liver

Shorr That is right

Engel If that is so the ferritin mechanism must become secondary

because it does not have anything to do primarily with the liver becoming hypoxic. Therefore, how can one distinguish between the abnormality in the liver with respect to inactivation of ferritin from any other metabolic phenomenon? What makes it any more important?

Shorr I should like to restate what you have said in this fashion The animal is exposed to a degree of shock which ordinarily affects the liver profoundly and which in particular damages the ferritin systems Regardless of what may happen to the over all oxidative metabolism of this organ, when we add an agent which preserves the ferritin systems, the administration of that agent and its effect on these systems are associated with survival. This doesn t mean that X, Y, or Z may not be the core of the problem instead of ferritin But so far we have no better evidence or specific indication of what goes wrong

Engel Is there any way you can more or less specifically poison the ferritin-inactivating mechanism in the animals protected with one of these drugs without knocking out a lot of other enzyme systems?

Fremont Smith Without hypoxia.

Engel Hypoxia presumably now is not important Apparently the liver is working all right

Baez Since the blood flow is better and the over all dynamics of the splanchnic capillary bed are unchanged in the pretreated animals, I wonder whether hypoxia develops in the liver to the same degree as in the controls

Engel It seems to me that the protective agents act by cutting down on the decompensating effect which would have resulted had ferritin been released In this case the critical experiment would be to devise some way of poisoning the particular mechanism for ferritin inactivation and showing that now the animal decompensates

Shorr We haven t done that. This agent is nontoxic in the doses we give The LD_{50} is about 75 mg/kg or 7½ mg/100 gm., and the effective dose is 80 μg/100 gm. or perhaps even less we have not tried lower doses

Recovery is associated with the administration of an agent which has no vascular effects that we can discern which is not adrenergic blocking is not anticholinergic, nor antibiotic, in fact has no pharmacologic effects in the animal as far as we know but which has a very definite effect on the ferritin systems in the liver The inference that we draw is that the persistence of the compensatory phenomena under these circumstances is a result of the liver s being prevented from releasing something which is deleterious to these compensatory reactions

Remington Dr Nickerson, is there a chance that this material would be converted, in the body into something which could act as an adrenergic blocking agent?

Nickerson It is hard to rule out such a conversion but I think it is most unlikely The body does not unsaturate saturated rings and the original conclusion that this agent lacks adrenergic blocking activity was based on *in vivo* studies in which the drug was administered to cats in maximum tolerated doses without reversal of the pressor response to epinephrine (2) We were quite conscious of the slow onset of action of members of this series Our acute intravenous experiments were run over a period of at least 6 hours and we also had some observations on animals injected subcutaneously for several days before testing

Shorr I should like to continue the ferritin story by describing rapidly some of Dr Mazur s more recent studies (5 6) Figure 43 presents the concept we held in 1950 of the activation and inactivation of ferritin Activation refers of course to its antidiuretic action as well as to its effects on the epinephrine reactivity of metarterioles and precapillary sphincters. We thought we could relate the activity to the sulfhydryl groups (7) When these were in the normal liver the ferritin state, the form in which ferritin exists in the normal liver the ferritin was inactive If they were reduced to the sulfhydryl form it became active

Fremont Smith May I point out that plus nitrogen really means minus oxygen?

Shorr Yes that is correct however there was one fact which did not fit in with this concept We could reactivate ferritin again after

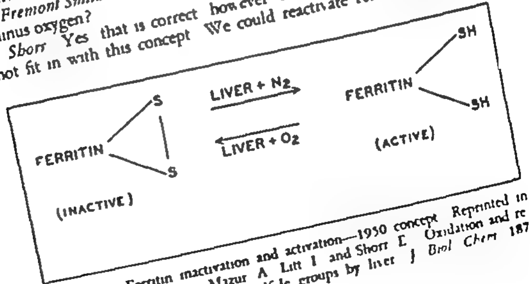

FIGURE 43 Ferritin inactivation and activation—1950 concept Reprinted in part, by permission from Mazur A Litt I and Shorr E Oxidation and reduction of ferritin sulfhydryl-disulfide groups by liver *J Biol Chem* 187 497 (1950)

it had been inactivated with iodoacetamide, which combines with sulfhydryl groups irreversibly Reducing agents such as cysteine and ascorbate were able to bring about this reactivation of iodoacetamide treated ferritin, but we learned that what changed under these circumstances was not the sulfhydryl but the ferrous iron of ferritin (Table XLV)

We fractionated ferritin with varying concentrations of ammonium sulfate and found that the fractions differed in total iron content but that the relationship between the ferrous iron and the total nitrogen content remained constant. Except in the fraction with the least total iron there was also a constant relationship between sulfhydryl and total nitrogen content and hence between the ferrous iron and the sulfhydryl These results are listed in Table XLVI.

We thought originally that iron was not important in the vasoactivity of ferritin because both apoferritin and ferritin were active though apoferritin has little if any iron (7 8) We now found (Table XLVII) that on anaerobic exposure of ferritin to liver slices in addition to the increase in vasoactivity and in sulfhydryl groups there was an increase in the ferrous iron and conversely on aerobic incubation with liver

TABLE XLV

Effect of Reducing Agents on Ferrous Iron Content of Iodoacetamide-treated Ferritin

Ferritin Treatment	$\frac{\text{—SH}}{\text{Total N}}$	$\frac{\text{Ferrous Iron}}{\text{Total N}}$
	μM/100 mg N	μM/100 mg N
(a) Original	16 5	2 0
(b) Ferritin + iodoacetamide	6 5	1 2
(c) (b) + cysteine	5.2	11 1
(d) (b) + ascorbate	5 7	4 9

Reprinted, by permission, from Shorr E. Intermediary metabolism and biological activities of ferritin. *Harvey Lect 1954 1955 112 (1956)*

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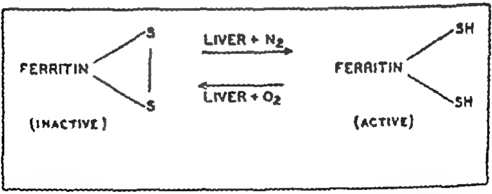
Fremont Smith May I point out that plus nitrogen really means minus oxygen?

Shorr Yes that is correct however there was one fact which did not fit in with this concept We could reactivate ferritin again after



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(b) Ferritin + iodoacetamide	6 5	1 2
(c) (b) + cysteine	5.2	11 1
(d) (b) + ascorbate	5 7	4.9

Reprinted, by permission, from Shorr E. Intermediary metabolism and biological activities of ferritin. *Harvey Lect 1954 1955 112 (1956)*

TABLE XLVI

Relationship of Total Iron, Ferrous Iron and Sulfhydryl Groups in Ferritin Fractions

Concentration of $(NH_4)_2SO_4$	$\frac{\text{Total Iron}}{\text{Total N}}$	$\frac{\text{Ferrous Iron}}{\text{Total N}}$	$\frac{\text{SH}}{\text{Total N}}$	$\frac{\text{Ferrous Iron}}{\text{Total Iron}}$
Per cent of Saturation	Micromoles per Millimole			
(Original)	(454)	(0.8)	(4 1)	(1 7)
0 to 27	549	0 7	3 2	1.2
27 to 31	454	0 6	3 3	1 4
31 to 34	361	0 6	3 4	1 6
34 to 50	251	0.8	5 1	3.2

Reprinted, by permission, from Mazur A. Baez, S., and Shorr E. The mechanism of iron release from ferritin as related to its biological properties. *J Biol Chem* 213 *147 (1955)*

slices along with the inactivation we found a decrease in both the ferrous iron and in the sulfhydryl groups

As we now began to study the significance of the iron rather than the sulfhydryl groups of ferritin we needed a more specific or direct way of removing ferrous iron from sulfhydryl ferrous-ferritin to find out whether or not the activity would be changed We found that *the plasma iron-binding protein has a very strong affinity for the ferrous iron of ferritin* The conversion of the ferritin iron to the ferrous form makes it more dissociable It is readily transferred across a membrane for combination with the iron binding protein of plasma The results of such an experiment are also given in Table XLVIII With the original ferritin prepared from horse spleen 17 per cent of the ferrous iron of the ferritin was bound by the plasma iron binding protein After incubation with liver slices in nitrogen the ferritin became even more active because as it was originally prepared it had not been completely reduced The sulfhydryl groups increased from 25 to 33 7 and the ferrous iron from 1 7 to 6 5 micromoles/100

TABLE XLVII

Effect of Liver Slices on Sulfhydryl Groups and Ferrous Iron of Ferritin

Ferritin Treatment	SH	Fe^{++} (c)	Fe^{++} Bound by Plasma Iron-binding Protein	
			Total	Per cent of (c)
	μM per 100 mg ferritin total N			
Original Ferritin	25.0	1.7	0.8	47
(a) Ferritin + liver slices in N_2	33.7	6.5	5.7	88
(b) (a) + liver slices in O_2	14.9	2.3	0.2	9

Reprinted, by permission, from Mazur A., Baez, S. and Shorr E. The mechanism of iron release from ferritin as related to its biological properties. *J Biol Chem* 213 *147 (1955)*

mg. of ferritin total nitrogen, and the iron bound by the plasma iron binding protein increased to 88 per cent The ferrous iron had become more dissociable. Conversely, when we oxidized the ferritin again, by aerobic incubation with liver slices, the sulfhydryl groups decreased to 14.9 micromoles/100 mg of ferritin total nitrogen, the ferrous iron content fell, and now only 9 per cent could be transferred to the plasma iron-binding protein

We found that the combination of surface ferrous iron of sulfhydryl ferritin with the *iron-binding protein* abolished its vasodepressor action and we infer from this that the ferrous iron is specifically related to this activity When we did the same thing to apoferritin, which presumably had no iron, we also made it inert, and we conclude that while the amounts of ferrous iron which had been retained in the apoferritin were too small for us to measure by our previous methods they could be removed by treatment with the plasma iron binding protein

Figure 44 represents the picture we now have of ferritin activation and inactivation. The ferritin molecule contains colloidal ferric phos-

TABLE XLVIII

Plasma Iron and Iron-binding Capacity (IBC) of Dog During Lethal Hemorrhagic Shock

Time Hr/min	Blood Pressure (mm Hg)	Rat Test*	Plasma Fe (µg/100 ml plasma)	IBC (µg Fe/100 ml plasma)	Saturation (per cent)
0/0	110	Neutral	158	190	45
0/20	90	VE(4+)	158	185	46
1/0	84	VE(2+)	179	193	48
1/35	67	VD(1+)	197	108	61
2/0	40	VD(2+)	180	95	65
2/40 (infusion of 40 ml blood)	40	VD(3+)	217	75	74
3/40	25	VD(4+)	333	25	93

* VE = vasoexcitor response given by dog plasma in rat mesoappendix test.
VD = vasodepressor effect.
(Dog No 295 13.6 kg Na pentobarbital 25 mg/kg died within 24 hr)

phate—ferric hydroxide micelles situated internally This is the bulk of the iron, but on the surface is a small amount of ionic iron which can exist in either the ferric or ferrous state It is the surface iron that is reduced by the anaerobic liver and we believe that chelation by the sulfhydryl groups helps to stabilize it Ordinarily ferrous iron added to proteins is readily autoxidized

We have talked so far only about changes in ferritin iron in the test tube The next step was to watch the changes in the plasma iron in animals going into shock We bled control dogs in the manner used in our laboratory to produce a high percentage of irreversibility Table XLVIII shows that during the early part of the experiment the

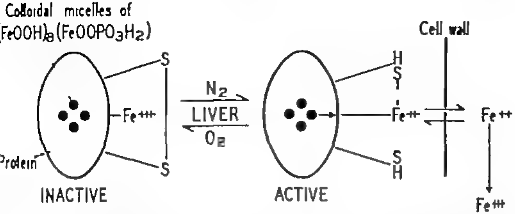


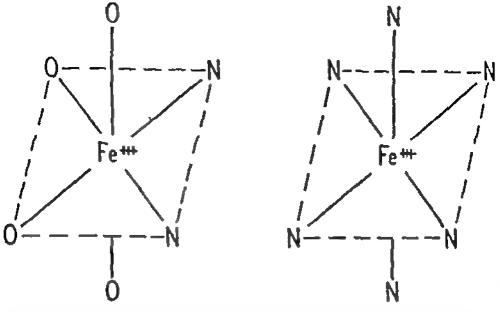
FIGURE 44 Ferritin inactivation and activation—1955 concept. Reprinted, by permission, from Shorr E. Intermediary metabolism and biological activities of ferritin *Harvey Lect 1954 1955 112 (1956)*

plasma iron changed very little, but when the blood pressure was reduced to drastic hypotensive levels there was an increase in the plasma iron content associated with a decrease in plasma iron-binding capacity and the saturation of the iron-binding protein rose to 93 per cent.

The liver ferritin systems were protected during shock in rats by dibenzyline pretreatment In a dibenzyline treated dog (Table XLIX) subjected to the same hemorrhagic procedure as the controls there was no increase in the plasma iron nor any drastic decrease in iron-binding capacity bio-assays of blood showed that ferritin did not appear in the circulation (9) In the untreated animal in irreversible shock, the ferritin stores of the liver are converted to the sulfhydryl ferrous form and the iron-binding protein of the blood is then able to remove quantities of iron which are sufficient to double or quadruple the plasma iron content. Table L summarizes these data

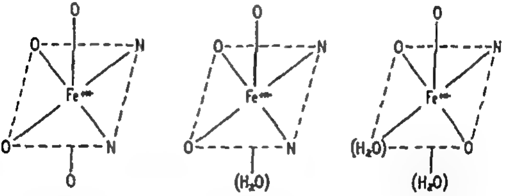
Dr Mazur and Dr S Green have just completed studies (10 11) which provide a possible explanation for the action of ferritin in inhibiting the constrictor response of the vascular smooth muscle cells to topical epinephrine in the mesoappendix test This action of ferritin may be related to the catalytic oxidation of epinephrine by ferritin iron. We postulate that the ferrous iron of ferritin on dissociation could enter into the cell where, no longer bound to SH groups it would be converted to the ferric form and be capable of catalyzing the oxidation of epinephrine to adrenochrome which in turn would be rapidly converted at neutral *p*H to vasoinactive melanin like compounds

These studies have revealed that epinephrine oxidation can be catalyzed by ferritin iron as well as by inorganic iron The chelating agent,



Fe^{+++}-EDTA $\qquad$ Fe^{+++}–Cytochrome C

FIGURE 45 Structural similarity of complexes formed by ethylenediaminetetraacetate (EDTA) and cytochrome *c* with Fe^{+++} Reprinted, by permission, from Green, S., Mazur A and Shorr E. *Mechanism of the catalytic oxidation of adrenaline by ferritin J Biol Chem 220 237 (1956)*



Isopropylene-diamine tetracetate $\qquad$ N-hydroxyethyl ethylenediamine triacetate $\qquad$ Nitrilo triacetate

FIGURE 46. Relation of activity as accelerator of catalytic oxidation of epinephrine by iron, to structure of complex Isopropylenediaminetetraacetate can furnish six co-ordinate bonds and is as active as EDTA Compound in the middle is less active and contains but five of the six groups required for binding to the iron atom. Compound at the right with four bonds is inactive Reprinted by permission, from Green, S., Mazur A., and Shorr E Mechanism of the catalytic oxidation of adrenaline by ferritin *J Biol Chem 220 237 (1956)*

TABLE XLIX

Plasma Iron and Iron binding Capacity (IBC) During Hemorrhagic Shock Pretreated with Dibenzyline

Time Hr/min	Blood Pressure (mm Hg)	Rat Test	Plasma Fe (μg/ 100 ml plasma)	IBC (μg Fe/ 100 ml plasma)	Saturation (per cent)
0/0	120	Neutral	96	190	34
0/30	65	—	116	158	42
1/0	52	VE(3+)	112	150	43
1/30	42	—	116	137	46
4/5	25	VE(2+)	110	120	48

(Dog No. 304 13.6 kg, 2 mg/kg dibenzyline 17 hr before experiment Na pentobarbital 30 mg/kg survived)

ethylenediaminetetraacetate (EDTA) was found to accelerate this action of inorganic iron and of ferritin iron. Instead of tying up the iron and reducing its catalytic activity as we had expected, EDTA made it more effective in oxidizing epinephrine We also learned that the most active chelating agents in this respect were the ones which contained the groups needed to form 6 co-ordinate bonds with the iron The configurations of cytochrome *c* and of chelating agents which have this property of accelerating the action of iron in the oxidation of epinephrine, are illustrated in Figures 45 and 46, as compared to those which are inactive in this respect Figure 46 shows the bonding of Fe^{+++} by isopropylenediaminetetraacetate which is as active as EDTA in augmenting the catalytic action of iron the middle compound is less active and the one on the right is inactive

I should like to go on to our most recent work which presents a different facet of this picture. About 21 years ago Peyton Rous (12) who is responsible for so many interesting things showed that if iron particles were injected in an animal, and after a period of time the liver were perfused the reticuloendothelial cells could be extracted from the perfusate by means of an eye magnet We thought this might help in the clarification of the sites of VDM (ferritin) formation and

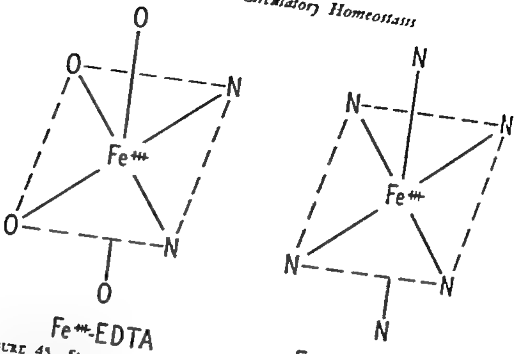
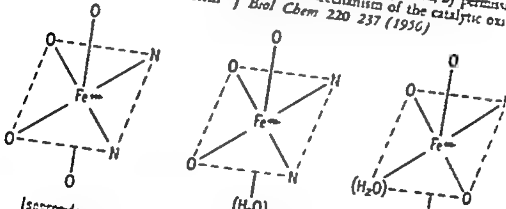


FIGURE 45 Structural similarity of complexes formed by ethylenediamine tetraacetate (EDTA) and cytochrome *c* with Fe^{+++}. Reprinted, by permission, from Green S., Mazur A., and Shorr E. Mechanism of the catalytic oxidation of adrenaline by ferritin. *J Biol Chem* 220 237 (1956)



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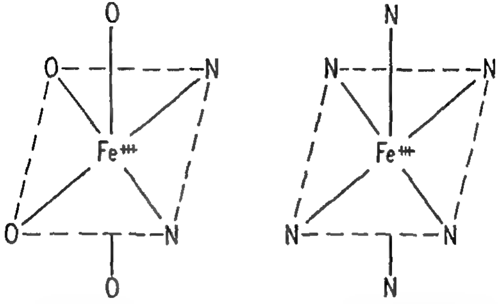

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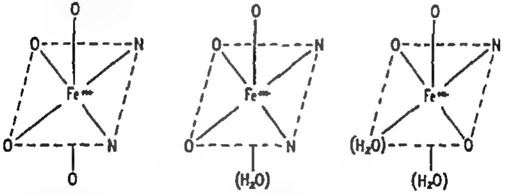

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TABLE L

Effect of Hemorrhage on Release of Iron Into the Plasma

Dog	Plasma Iron (last sample)	Iron-binding Capacity (last sample)	Saturation of iron binding protein (last sample)
	Per cent of the Original		Per Cent
Controls (died)			
387	377	6	97
343	534	7	94
295	210	13	93
330	---	13	--
389	193	19	92
294	---	14	--
336	225	10	88
353	173	24	83
382*	300	25	80
Controls (survived)			
362†	380	10	93
388	216	47	81
380	165	39	75
383	159	58	65
364	142	38	55
Dibenzyline-treated (survived)			
361	150	54	67
304	115	63	48
301	---	79	--
365	114	100	10
358	50	133	27
390	70	85	18

Last blood sample was lost, figures are based on the last sample analyzed

†This dog survived beyond 24 hours but died subsequently

inactivation Figure 47 *A* is a photograph of a group of reticuloendothelial (Kupffer) cells that have been so separated. Carbonyl iron with particle sizes of from 2 to 5 μ was injected into a rat After about 48 hours the liver was taken out, put through a garlic press, and then sieved through silk (13) The filtrate was suspended in an albumin Ringer phosphate medium The reticuloendothelial cells could then be drawn down with a magnet the supernatant parenchymal cells decanted, and after several repetitions the reticuloendothelial cells were entirely separated from the parenchymal. These were still viable cells, they breathed

Engel Can you get the iron out of the reticuloendothelial cells?

Shorr Yes if you homogenize the cells but at present we use them in this fashion Intact parenchymal cells which are without iron are shown in Figure 47 *C*

Figure 48 shows that when the separated cells were incubated in nitrogen VDM activity was found only with the reticuloendothelial cells and not with the parenchymal cells

The reticuloendothelial cells which released the VDM anaerobically could not inactivate it aerobically whereas the parenchymal cells which did not release it, were able to inactivate it. This should be very helpful in the search for an enzyme system or systems responsible for activation and inactivation

Furchgott Which of the types of cells has the ferritin to begin with?

Shorr Only the reticuloendothelial cells released VDM activity

Furchgott There is none in the parenchymal cells?

Shorr None that is measurable by the mesoappendix test *

Fremont Smith It is important to keep in mind the fact that the iron that is put in is merely a method of separating the cells and that iron plays no role in the ferritin

Shorr Carbonyl iron alone was neutral in the mesoappendix test. These are the directions in which we are currently working

Burton May I ask if ferritin is not ferromagnetic itself?

Shorr Yes but it would not be possible to pull it down with a magnet there is not enough activity We tried, without success

*Results obtained subsequent to the meeting: When immunochemical tests were applied to the clear supernatant solution after homogenization of the separated cells, contrary to our expectations, the parenchymal cell supernatant formed a brownish precipitate with anti-ferritin serum, indicating that it contained ferritin. Repeated mesoappendix bio-assays of anaerobically incubated parenchymal cells over a wide range of dilutions consistently were neutral. However when a little of the clear supernatant homogenate of reticuloendothelial cells was added to the parenchymal cells, VDM activity appeared on anaerobiosis. This amount of anaerobically incubated reticuloendothelial homogenate was in itself without activity in the mesoappendix test. Our present inference is that the parenchymal cells contain ferritin which, however remains inactive even on anaerobic incubation, unless accompanied by a factor or activator derived from the reticuloendothelial cells.

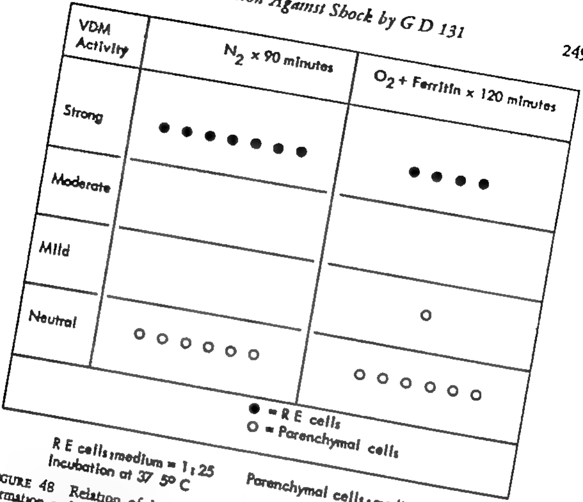

FIGURE 48 Relation of hepatic parenchymal and reticuloendothelial cells to formation and inactivation of ferritin. VDM activity abolished by antiserum, hence due to ferritin.

Baez. With samples of homogenized liver

Burton Dr Pappenheimer Sr told me that the malarial infected red cells can be separated out by a magnet because of some metabolic change in them.

Shorr Is there ferritin in them?

Burton I do not know what it is

Shorr We found that without the injection of carbonyl iron the effort was fruitless

Rappaport Dr Shorr have you ever tried to put these ferritin containing cells into a magnetic field to see if they arrange themselves in lines?

Shorr No we have not

Rappaport This is not my idea it is Peyton Rous s (12)

Burton Also if there is ferritin in the blood even though only

a minute amount were magnetic, it would be possible for a radio-physics man to make an apparatus which would show the inductance between two coils if a slight magnetic permeability caused by the ferrous material was present it should show up very well.

Shorr In the blood in severe shock the ferritin content probably never rises above about 0 002 μg of ferritin N per ml That would give very little magnetic activity

Barton The method I am suggesting is enormously sensitive.

Shorr This is what we have been working on just now and it is obviously indicating many types of experiments which must be done.

Knisely Have you published your method for separating the two types of cells?

Shorr No but we have modified slightly the method of St. George, Friedman, and Byers (13) which was based on Peyton Rous s idea (12)

Knisely Their method gives an opportunity to separate all of the known and as yet unknown metabolic processes of Kupffer s cells from hepatic parenchymal cells

Shorr As I said we have done this only recently It has been an exciting development which has possibilities for investigation of iron deposition and transport, etc. There are many experiments to be done.

Engel I do not quite understand EDTA In what is EDTA used?

Shorr We were studying the catalytic oxidation of epinephrine to adrenochrome by inorganic iron and by ferritin iron We found that chelation by ethylenediaminetetraacetate, EDTA, greatly accelerated the reaction, instead of making the iron ineffective as we had expected. This led to a study of other chelating agents and we found that only those are active which, like cytochrome *c* yield a structural arrangement with iron similar to that of EDTA and iron In enzymes which accelerate oxidation the inorganic metal ion has been thought to be the important thing our studies point to the importance also of the structural arrangement

Engel In your opinion, would this eliminate the possibility of using a chelating agent to take out the iron that is in the blood in shock?

Shorr No since the effect we have noted, the acceleration of epinephrine oxidation by iron and EDTA is so far limited to chelating agents which form configurations similar to that of EDTA with iron Others may very well bind and inactivate iron without the accelerating effect of EDTA

The significance of the EDTA acceleration lies instead, in the suggestion that normally occurring compounds in the smooth muscle cell may combine with iron (from ferritin) to form extremely active complexes which would oxidize and inactivate epinephrine For example,

we found that cytochrome *c*, a biochemically important iron-chelate, could serve to oxidize epinephrine more actively than an equivalent amount of inorganic iron.

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INFLUENCE OF HYPOTHERMIA CHLORPROMAZINE

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I SHOULD LIKE to discuss some of our recent observations on the use of hypothermia and chlorpromazine in experimental hemorrhagic shock

We were stimulated to do this work by the concept of the French workers who have termed it artificial hibernation and who have made rather broad claims for its value not only in the experimental animal but also clinically as a means of protection against the state of shock

However significant this French work may be, it seemed to us it was somewhat obscured by the polytherapy which they employed. It was our thought that perhaps we might be able to obtain more precise information if we applied the methods and agents that they used to the preparation of experimental hemorrhagic shock which is essentially similar to that described by Dr Fine. We had had considerable experience in the laboratory with the application of aureomycin to this form of shock I do not wish to go into the details of this procedure, but it may be of value for me to describe briefly the technique of the preparation

We used mongrel dogs, some of which must have had apparently low levels of properdin None the less they were comparable animals in terms of the controls and they averaged about 14 5 kg in weight They were bled via the femoral artery into a reservoir arrangement until the blood pressure reached a level of about 30 mm Hg Then the animal was allowed to remain at that level. As soon as there was evidence that the compensation mechanism failed blood was returned to the animal until 40 per cent of the total amount of blood removed initially had been returned to the animal this constituted the first end point.

The second end point came at the end of 8 hours of hypotension at the level of 30 mm. Hg if 40 per cent had not been restored In

other words, this is essentially similar to the technique Dr Fine has described and which we had used previously We felt that we should continue this technique whether or not it had certain disadvantages, because we had had considerable experience with it, and with this background of experience we could evaluate better the experimental procedures that were employed.

There were five groups of experimental animals in addition to the controls One experiment and a control were done at the same time, occasionally we would run two experiments simultaneously with one control

In one experimental group hypothermia alone was used This was done by wrapping the animal in a rubberized blanket through which coils of refrigerant solution circulated By this means the rectal temperature was reduced to 31°C. in approximately an hour The experiment was then continued in a manner essentially similar to the procedure used in the control

Nickerson Was the cooling carried out without anesthesia?

DeBakey No In the previous experiments with the aureomycin treated dogs, we had used local anesthesia just as Dr Fine did But because the cooling procedure produces shivering which we wished to avoid, we used general anesthesia with morphine and phenobarbital given intravenously

The second group of animals was treated with chlorpromazine alone in dosages of 50 mg and 100 mg given intramuscularly 1 to 2 hours before bleeding The difference between 50- and 100-mg dosages as used here has no statistical significance, both being large doses for the dog A third group of animals was treated with chlorpromazine alone in dosages of 5 mg A fourth group of animals was treated with the combined procedure of cooling plus chlorpromazine at dosages of 50 mg and the fifth group with combined cooling plus chlorpromazine in dosages of 5 mg In treating these groups every thing else was kept constant and similar to the controls as far as it was possible to tell

Figure 49 shows that the control group had an immediate 24 hour high mortality 96 per cent, a fairly constant figure among our controls using this preparation. In our previous study using aureomycin-treated animals the immediate mortality among the controls was slightly less being closer to 90 per cent It is important to remember however that the general anesthesia used in the present experiments represents another possible stress factor

Fremont Smith To what extent did you cool the animals?

DeBakey We cooled them to 31°C. Figure 49 shows that there

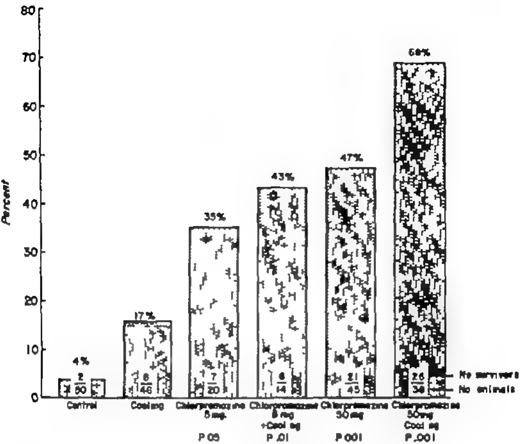

FIGURE 49 Immediate survival in experimental hemorrhagic shock. Reprinted, by permission, from Overton, R. C. and DeBakey M. E. Experimental observations on the influence of hypothermia and autonomic blocking agents on hemorrhagic shock. *Ann Surg* 143, *439 (1956)*

was an increase in the survival rate in all of the experiments as compared with the control animals We also repeated this experiment using exactly the same groups, and found that the ultimate survival rate at the end of 2 weeks after the operation was also increased This period was chosen because we felt that if at the end of 2 weeks the animal appeared to be perfectly normal and restored, there was no use in feeding it any longer

Figure 49 shows that there is a fairly significant protection in terms of survival rate with the use of chlorpromazine Cooling plus chlorpromazine gave the most significant and the highest survival rate This was really quite striking because up until this time we really had never been able to affect the survival rate in this preparation by any other means

In our earlier studies with aureomycin we felt that although there was a slight increase, actually the survival rate was not significantly

increased. We were beginning to feel that this type of preparation was a highly fatal one and that very little, if anything could affect it, until we ran into the phenomenon which is shown in Figure 49

TABLE LI

Experimental Hemorrhagic Shock

	Initial Blood Pressure mm Hg	Time to Reach Maximum Bleeding Volume	Maximum Bleeding Volume ml/kg
Control	93	1 hr	51.8
Hypothermia	94	1 hr 18 min	51 4
Chlorpromazine 50 mg	80	3 hr 24 min	41 1
Chlorpromazine 5 mg	84	2 hr 24 min	52 1
Hypothermia + Chlorpromazine 50 mg	73	3 hr 51 min	42 4
Hypothermia + Chlorpromazine 5 mg	90	2 hr 32 min	53 5

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In addition to the evidence that agents, *i e*, cooling and chlorpromazine, have a strikingly beneficial effect in providing the animals with greater ability to tolerate this stress procedure, there are some additional data which suggest support of these beneficial effects

Shorr Dr DeBakey, at what time did you give the chlorpromazine in relation to the shock?

DeBakey Approximately an hour before

Fremont Smith How long did it take to cool the animals?

DeBakey About an hour

Fremont Smith So the chlorpromazine was given about 2 hours before the lowered blood pressure?

DeBakey That is right The cooling took about an hour The chlorpromazine was given an hour before that Then the animals were bled That would average about 2 hours

Figure 50 shows the percentage of animals in each group that tolerated the hypotension for ▮ hours when the 40 per cent end point was reached It is evident from the figure that there is a high proportion of tolerance to hypotension among the animals treated by the combined use of hypothermia and chlorpromazine.

Figure 51 represents the average hypotensive time for each group *i e* the average duration of hypotension including the animals that were arbitrarily terminated at 8 hours Here again there is a similar arrangement in terms of the progressive increase in the tolerance of the experimental animals to this hypotensive period as compared with the controls

Fremont Smith The end point is that point when 40 per cent of the blood had been taken back by the animal?

DeBakey That is right.

Fremont Smith It is the same thing looked at from a different angle

DeBakey Exactly Table LI shows the initial blood pressure in these animals the average time required to reach the maximum bleeding volume before we started returning blood, and the maximum amount of blood removed in ml per kg Here is evidence that we have changed the experiment by the use of chlorpromazine The controls and the hypothermia are fairly comparable in terms of initial blood pressure and time to reach the maximum bleeding volume as well as in maximum bleeding volume per kg There is not a great deal of difference between the controls and the hypothermic animals We really did not change the experiment greatly by applying simple hypothermia, by just reducing the body temperature to 31°C. However when we applied chlorpromazine at dosage of 50 mg we made a significant difference in terms of the time to reach maximum bleeding

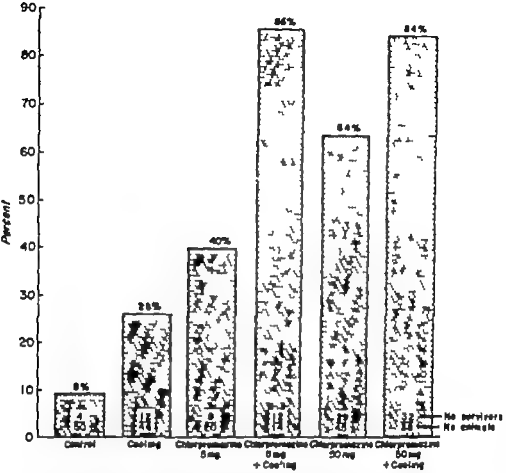


FIGURE 50 Experimental hemorrhagic shock. Number of animals tolerating hypotension 8 hours. Reprinted, by permission, from Overton, R. C., and De Bakey M. E. Experimental observations on the influence of hypothermia and autonomic blocking agents on hemorrhagic shock. *Ann Surg* 143 439 (1956)

volume In other words the animals tolerated the hypotension much longer and the amount of blood removed was less so that in a sense, we have altered the experiment we have introduced a factor which changes the experiment and makes these animals start off at a different level than the controls They started with lower blood pressure then bled more slowly and did not bleed out as much during the hypotensive period I feel that this constitutes a significant factor in the results obtained I would appreciate suggestions as to how we can overcome the introduction of another variable by the use of this agent

Nickerson Do you know how much chlorpromazine is required to produce significant adrenergic blockade under these conditions?

DeBakey No I do not

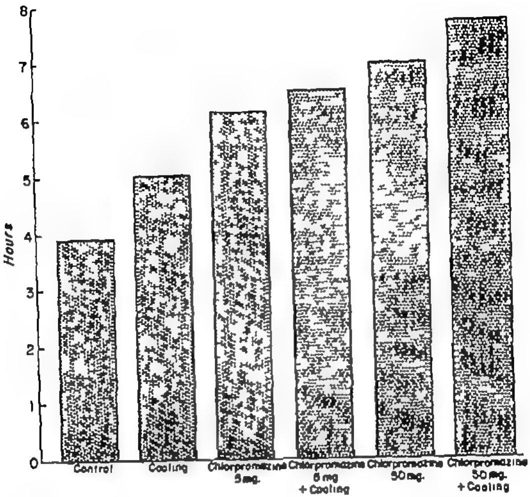


FIGURE 51 Experimental hemorrhagic shock. Hypotensive time Reprinted, by permission, from Overton, R. C., and DeBakey M. E. Experimental observations on the influence of hypothermia and autonomic blocking agents on hemorrhagic shock. *Ann Surg* 143 *439 (1956)*

Nickerson The adrenergic blockade produced by this agent is quite variable under different circumstances but in some experiments doses of 1 0 mg /kg or less can be shown to have a significant effect

DeBakey It is very interesting to note that among the animals receiving only 5 mg of chlorpromazine the maximum bleeding volume was essentially the same as the controls, but the time to reach maximum bleeding volume was increased

Knisely Dr DeBakey how many animals are in each group?

DeBakey In the controls, there are 50 animals in the hypothermia alone, 46 animals in the 5 mg of chlorpromazine, 20 animals, in the 5 mg of chlorpromazine plus hypothermia group 14 animals in the

reduce the pressure Our criterion of maintaining the animal at a certain level of arterial pressure is no longer in operation

Nickerson Your data on the different doses of chlorpromazine are very similar to those with the adrenergic blocking agents

DeBakey That is right

Nickerson With small doses the bleeding volumes are essentially equal to those of the controls, but with high doses the standard pressure is maintained with less bleeding

Baez We think that depends to a great extent on the way the animal is bled With a slow graded type of hemorrhage our dibenzyline treated dogs and rats (2 3) shed equal or slightly larger amounts of blood than their controls and yet more of them survived.

Nickerson Rate of bleeding is important, but even with your type of bleeding, if you gave 50 mg /kg instead of 2 mg /kg of dibenzyline, you would probably find that the bleeding volume was reduced The key experiments in any of these series are the ones where a dosage has been used which changes the survival rate without changing other variables

DeBakey That may well be the most significant feature of the experiment

Rappaport I should like to ask about the control animals Have you seen such low blood pressure in other experiments with dogs? I know from our experiments that dogs in pentobarbital anesthesia usually have a blood pressure between 120 mm. Hg and 140 mm Hg

DeBakey These are average blood pressures for the controls

Fremont Smith Where are your blood pressures measured?

Rappaport In the femoral artery

Selkurt I wanted to ask the same point for the same reason, on 120 130 140 mm Hg

DeBakey We used 10 mg of morphine sulfate/kg and 15 mg of pentobarbital/kg given intravenously

Rappaport It might be the morphine that is making the difference

Selkurt Yes

Barton The arterial pressure would be definitely lower with morphine

Nickerson I think it is clear that the morphine is not inert This applies also to Dr Fine s experiments although he referred to the morphinized animals as "unanesthetized The dose of morphine per kilogram of dog is equal to the total average human dose

Dobson We found that morphine decreases liver circulation considerably too In regard to morphine we found that a dose of 3 to 6 mg /kg reduced the total liver blood flow to about three fourths

of normal Our method measures total hepatic flow and does not distinguish between hepatic artery and portal vein flow

Furchgott I should like to bring up one more pharmacologic point in connection with chlorpromazine which might have a bearing on your bleeding volumes Chlorpromazine when tested on cardiac muscle preparations *in vitro* causes a slight depression of contractile force at about one part in a million and a considerable depression at one part in a hundred thousand I believe that 50 mg /dog may be approaching a range where there is some small direct effect on the heart It may not influence the final outcome adversely but it might account for some of the reduction in bleeding volume

DeBakey Yes that could be

Green Did I understand that your chlorpromazine animals have a degree or two lower temperature than the controls?

DeBakey Yes

Green Did you attempt to control their temperatures by covering the animals?

DeBakey No

Green Even a degree or two of temperature might make a striking difference in survival rates

DeBakey Yes that is certainly true That has occurred to us, and we are going to try to check for it We will try to keep the body temperature the same

Green Did you pair your control and chlorpromazine animals and use paired animals at the same temperature?

DeBakey Yes All we do is apply body warmth to the animal

Green From your present data could you pick out pairs of animals?

DeBakey I should think that would be a dangerous thing to do I should not want to rely on data of that kind I doubt that we could pair them that way because they all showed slightly lower temperatures When chlorpromazine was given to an animal in a dosage as large as 50 mg the temperature was reduced on the average of one degree and up to as much as two degrees in some instances However the 5-mg dosage tended not to lower temperature The only way we can control that, of course would be to apply warmth to the animal and keep the body temperature up We did not do that but it may be a worth while procedure

Nickerson Was there no fall in the body temperature of the control animals at your somewhat high room temperatures?

DeBakey Very slight

Nickerson At an ambient temperature of about 70 F there may be a significant fall

DeBakey That is right As I indicated earlier the average temperature in the laboratory will run between 76° and 78°F and occasionally it will rise as high as 80° F with four or five people in the room, as there are for some of these experiments which run 8 or 10 hours The experiment might be started at 76°F and by the time it is finished the temperature might be close to 80°F That tends to prevent the lowering of the dog s temperature that ordinarily would occur if the room temperature remained low

There is one other observation I should like to add This does not bear directly upon the problem, but it may have some significance We became interested in the effect of systemic infection in the presence of hypothermia I might say that the reason we became interested was because of certain clinical observations in the use of hypothermia and the occurrence at least in one case and subsequently in a few others of very severe infection that could not be controlled by any chemotherapeutic means The first instance we observed had an infection which we thought was related to the presence of a traumatic aneurysm of the arch of the aorta We felt that it was imperative that we remove the aneurysm otherwise we could not really control the infection The patient was placed under hypothermia during the interruption of circulation through the aorta in order to protect the spinal cord from damage This was done quite satisfactorily and successfully and the patient recovered from the operation and lived about a week. He was getting along quite well from that standpoint but had all the evidence of septicemia and bacteremia We were unable to control the bacteremia by any means that we employed

Tremont Smith Had he had antibiotics before the cooling?

DeBakey Yes We knew beforehand that the patient had the infection which we thought was from the aneurysm The aneurysm proved to be infected

Tremont Smith I meant the resistance of the organism to the antibiotic might have taken place before the cooling

DeBakey It very well might have, but after the cooling it was completely resistant We thought possibly that the change may have occurred during the hypothermia

Then we examined the literature carefully in an effort to find something on this problem but we could not There is some work that was done about 12 or 15 years ago (4 5 6) on the effect of local body cooling on local infection but not on systemic infection. We attempted to work on this problem in the laboratory and found that when we injected for example a pure culture of hemolytic *Staphylococcus aureus* into a group of animals there resulted a preparation that would give a 10

per cent mortality We then planned to apply hypothermia to these animals but realized that in order to do so we had to use anesthesia For this reason it was necessary to determine the effect of anesthesia upon infection Accordingly two additional groups of animals were studied, one in which anesthesia and hypothermia were employed without infection and one in which anesthesia plus infection was used The mortality in the former was 10 per cent and in the latter 30 per cent This is to be compared with the control animals in which only infection was used with a mortality of 10 per cent Thus the addition of anesthesia increased the mortality from infection from 10 per cent to 30 per cent. In still another group of infected animals hypothermia was used anesthesia was of course also used in these animals and the mortality was 50 per cent It is thus apparent that the addition of anesthesia (barbiturate) increases the stress in these animals and adversely affects the infection

Aloe What is the mechanism of death in the animals that died of hypothermia alone?

DeBakey They varied somewhat Most of them died of cardiac arrhythmias and fibrillation.

Aloe During hypothermia?

DeBakey Yes

Aloe Not during the warm phase?

DeBakey Sometimes during the warm phase, but before they reached the normal body temperature.

Furchgott Did you ever run into this type of death with chlorpromazine plus cooling?

DeBakey It would be hard to say We had an occasional death but we felt in some instances it was probably a technical error of one kind or other that is that the cooling was done too quickly and that we precipitated the occurrence of fibrillation It did not occur once the experiment was under way

Furchgott The reason for my question is that certain workers, including some of our medical students have shown chlorpromazine to be an excellent antagonist against arrythmias caused by epinephrine or by epinephrine plus chloroform or petroleum ether I would therefore suspect that deaths caused by fibrillation would not be nearly so frequent in dogs treated with chlorpromazine

DeBakey No it really did not occur in any significant degree We disregarded its occurrence

Nickerson The problem of arrhythmias during body cooling is rather complex Below your temperature range where the arrhythmias become the primary cause of death adrenergic factors do not appear to

be involved in their etiology, at least the potent adrenergic blocking agents provide little protection in this temperature range

DeBakey That is true I think that is one of the reasons we really did not encounter this problem as a serious one The body temperature of 31°C. is not a very low one

Baez. Is it correct that there seems to be a critical temperature at about 28°C., where most of the arrhythmias are observed and below that level at 24° or 23°C. none occurs?

DeBakey I must say this has been confirmed in our clinical experience, which is not great but fairly appreciable. We have not encountered serious arrhythmias at body temperatures above 86° or 87°F In fact we are now finding we can do essentially what we need to do at body temperatures slightly below 90 to 91°F Most patients, even those over 60 years of age tolerate this degree of body hypothermia. Apparently this temperature still has a protective effect against ischemic damage

Selkurt Have you tried the measurement of oxygen utilization in dogs and compared the 31° C. group with those at normal body temperatures?

DeBakey No

Shorr It would be interesting to work on the low side wouldn t it? There are considerable data on the high side in patients with typhoid fever

Selkurt Yes of course

Fremont Smith We had a recent conference here on cold injury Dr Andjus of the University of Belgrade and Dr Audrey Smith, of the National Institute for Medical Research, London, reported on their experiments in cooling of whole animals rats hamsters and monkeys down to solid freezing (7) According to their calculations they freeze to ice some 60 per cent of the animals body water After rewarming the animals seem quite normal Dr Smith was even able to cool pregnant hamsters to solid freezing They were super supercooled and were so solid that they would break if struck She could rewarm them again, and they would give birth to normal offspring, provided they were not cooled between the ninth tenth or eleventh day of the 16-day pregnancy If they were cooled at that time then the newborn animals had all kinds of congenital defects If they were cooled before those days or after the twelfth day there seemed to be no ill effects

I do not think this is exactly pertinent to this discussion but I thought some here to whom this was not known would be interested We saw movies of this and it is a very dramatic thing The respiration ceases, the heart ceases and all brain electrical activity that it was possible to

measure ceased. Then all of these functions seemed to come back again

Engel Was the cooling rapid or slow?

Fremont Smith Quite rapid. Another thing that was very interesting was that rats which had learned to run a maze and which were super cooled and then rewarmed, still remembered the maze. It was only the rats that had a very brief cooling that had a memory loss which is interesting because again it is usually most recent memories the ones that have resided the shortest time in the central nervous system that are the most vulnerable. This seems to be a general law as far as I can make out

Hasst This was rapid cooling in a certain sense only It was not rapid as compared to cooling with liquid air

Fremont Smith Quite right the temperatures were lowered in 3 or 4 hours

Baez They begin the cooling experiment by placing the rat or guinea pig in a covered beaker in a refrigerator After a certain time the animal is unconscious and quite cool Then they proceed with further cooling

Dobson This precooling in the ice box I believe was not necessary with the hamsters and the natural hibernators

Burton It is being found that is is not necessary in any of the animals

Fremont Smith It was just the way the experiment happened to get started The hamsters can warm themselves up by shivering the rats have to be brought up to and kept at normal body temperature for 24 or 48 hours before they regain their temperature control

Burton To me the most remarkable thing in that conference was the evidence that Dr Andjus had of a biologic adaptation He would cool the rats repeatedly and after they had been through this procedure once the second time they were much more resistant and the third time even more so

Fremont Smith You mean they tolerated the cold better?

Burton Yes This was really a remarkable thing that he could train them so that they stood the cooling very easily indeed

Dobson Just like drum shock

Shorr Dr DeBakey have you any idea how chlorpromazine brings about such high protection in shock?

DeBakey I really do not have any good ideas as to how it operates, but I am inclined to believe that it does so at least in these animals I am not prepared to go beyond what we have observed and apply it to humans As far as the animals are concerned I am inclined to

believe that under the conditions in which this experiment is performed we are getting increased survival rate primarily because we are altering the experiment at least to some degree. Even with the use of a small dosage of chlorpromazine where it would appear that the experiment is changed very little the fact remains that we prolong the hypotensive time, although the maximum bleeding volume was essentially the same In other words I have a feeling that in some way we are reducing the stress and that there is a margin perhaps not a great one, but never theless a margin, which may be changed sufficiently to allow a certain proportion of dogs to survive

Fremont Smith When you say that if you do not define the stress or its reduction, all you are saying is that the animal has survived longer and you are not introducing any concept or mechanism involved When you say you reduced the stress this is defined by the fact the animal urvived Won t you elaborate on this a little?

DeBakey One can conceive of the possibility that the organism is able to tolerate a certain amount of stress Beyond that comes a point in any given animal where he is unable to tolerate it no matter what is done after that time he has reached his maximum of tolerance But if you move that point by doing anything to him by in this case chlorpromazine he will tolerate the stress a little better Obviously there must be some difference between one animal and another in reaching the point of intolerance In some animals we are able to change the point with chlorpromazine

Fremont Smith You are saying that if the animal arrives at a certain point, no matter what is done he is going to die but that if the stress is reduced, he will live and that giving chlorpromazine has reduced the stress I should like to put this the other way and say that here is an animal that, no matter what is done will die but something was done to him and he did not die I want to know what you did to him and why he did not die You say you reduced the stress?

DeBakey I do not know what I did

Fremont Smith Isn t there a mechanism involved that we can approach?

DeBakey That is the thing I am not able to get at except what the data suggest and they suggest that chlorpromazine changes the experiment in terms of the amount of hypotension that the dog tolerates It allows him to have a longer period of hypotension rather than the average period Instead of his being in hypotension for say an average of an hour and then dying he now lasts 2 hours

Fremont Smith The longer period of hypotension should be all the more reason for his dying

DeBakey No I do not think so

Burton Would you say he has a larger blood volume for a longer time? The final bleeding volume may be the same He had that extra blood volume a little longer

DeBakey He keeps the extra blood volume a longer period of time

Nickerson I do not think that is quite correct Dr Burton The slides shown indicated two differences between the control animals and those given the lower dose of chlorpromazine One difference is that it took the treated dogs longer to reach their maximal bleeding However the total time for reinfusion appeared to be increased as much as the length of time to maximal bleeding The dogs treated with the chlorpromazine were at their minimal blood volume for as long as the controls It appeared to me that the two factors essentially balanced each other

Burton You might try heating and ventilating people who describe the climate in terms of the hours below a certain level, *i.e* as the product of the days times the deficit of temperature below that level In other words you might try analyzing these data in terms of milliliters deficit of blood volume, times hours or times minutes, and see if this was a factor which correlated with the survival

Nickerson Dr DeBakey actually has presented such data, although he has not calculated the units Gross inspection of his figures indicates little difference in the two groups However we might say that the treated dogs have been subjected to less stress simply on the basis of the fact that they survived.

Green There is less stress in terms of blood volume removed but more stress in terms of hours

Fremont Smith He has not defined the stress

Nickerson I should like to suggest the nature of this reduced stress The stress is not less in terms of the amount of blood removed or the duration of hypovolemia The difference in the stress to the organs of the treated and control animals is related to the way in which the residual blood is circulating I should like to suggest that the chlorpromazine has reduced vasoconstriction in the treated animals and that their organs are subjected to less stress because the residual blood is circulating more effectively

Dobson I think that is very important

Kinsely I wanted to say the same thing in a different way You talked about the arterial pressure, but two other factors might also be interesting One is the cardiac output and the other is *total oxygen* consumption

Baez I should like to interject the following information which

might have a bearing on the point under discussion A single injection before the experiment, of 50 mg of chlorpromazine (contained in the lytic cocktail) has been reported (8) to give significant protection in anesthetized dogs against a different kind of shock, that caused by drum trauma

Nickerson It is reasonable to speculate, in connection with Dr Knisely s comment, that both the cardiac output and the oxygen utiliza tion would be better in the treated than in the control animals Both of these are increased by adrenergic blockade even when the amount of hemorrhage is the same as in the controls

Selkurt In vital organs, especially the heart, brain liver and kidney, there is the possibility of shunting so that the same cardiac output or blood volume could be more effectively used where it ought to be used From the standpoint of acute need, we might leave out the kidney and say that brain, heart and liver probably are the more important organs

Remington You still will not put muscle in the list of important organs?

Selkurt During shock the animal would largely cease using its skeletal muscle which would not be as important as the organs dealing with vital metabolic phenomena

Shorr I should leave in the kidney

Nickerson Perhaps Dr Selkurt s list plus some rather preliminary work done with Dr Lloyd Beck at the University of Michigan will tell us more exactly what organ is involved in the development of irreversi bility Our preliminary results suggest that the brain and heart are relatively unimportant In these experiments a catheter was passed down the aorta, and a balloon on the end of it inflated so that the anterior part of the animal remained normotensive while the posterior part was bled to a pressure of from 40 mm Hg to 45 mm. Hg Irrevers ible shock appeared to develop in essentially the same way when only the posterior part of the animal was hypotensive as it did when the whole animal was involved

DeBakey In that connection we made some observations on human beings that may have some interest to the group in connection with the blood flow and the tolerance of particularly the liver and the kidney relative to ischemia. In certain types of conditions such as aneurysms involving the thoraco-abdominal aorta we now have performed four operations for resection and homograft replacement of the involved segment of the abdominal aorta This segment includes the branches of the celiac artery which of course gives rise to the hepatic and splenic arteries the superior mesenteric artery and both renal arteries In performing this procedure it was necessary to occlude the aorta above

and below the aneurysm and temporarily arrest circulation through these major abdominal visceral branches During period of occlusion blood flow was arrested for example, for well over an hour in the celiac axis so that the liver and kidneys were deprived of blood for about an hour The superior mesenteric artery which of course supplies the intestinal tract, was occluded for anywhere from approximately 50 minutes to an hour and a half The kidneys were deprived of blood for a period ranging up to 33 minutes Liver and renal function studies were done on these patients before and after operation. As far as the gut is concerned it appears to be able to tolerate this period of ischemia without any difficulty because all these patients had complete restoration of gastrointestinal function

The liver function studies which were very gross but which nevertheless are the function studies usually done clinically showed no significant changes. There was some depression early but in time apparently none.

The most significant effect occurred on the kidneys This effect was one of depression in total urine production to as little as 2 and 3 ml /hour for periods ranging up to 3 or 4 days but never true anuria The urine flow gradually rose over a period of several days and returned to normal within a period of a week But glomerular filtration rate and related kidney function studies indicated that kidney function was markedly depressed so that the kidney was virtually putting out only water for the first week. Following this there was a gradual restoration and within 2 weeks the kidney function had returned to better than 50 per cent of normal

Selkurt I missed whether these were hypothermic or normothermic

DeBakey Three were normothermic one hypothermic

Shorr Were they treated with antibiotics?

DeBakey Penicillin and streptomycin, and subsequently in the post operative period we added acromycin

Shorr Then possibly some protective effects may have been exerted by the antibiotics

DeBakey Yes

Fremont Smith Was there any drop in blood pressure when you allowed the circulation to return through the liver?

DeBakey There was a moderate drop

Fremont Smith That is what would be anticipated

Selkurt We found a marked drop in arterial pressure upon restoring flow after about 2 hours of ischemia of the liver in the dog

Shorr Dr Selkurt I have been wondering why none of the surgeons

has followed your procedure of an external circuit for the portal flow (9) to protect the gut. Would that be too damaging?

Selkurt Don t they perform an Eck fistula to permit venous drainage of the intestine?

Shorr Do you create an Eck fistula?

DeBakey In the patient I described, no

Shorr Perhaps some of the difficulties from interrupted blood flow could be overcome by Dr Selkurt s procedure.

Selkurt His situation is different He closes off the arterial inflow so that an Eck fistula is not so important here.

Fremont Smith Dr DeBakey your experiment with the kidneys is a bit surprising If I understand in some of the experiments the kidneys were badly hurt after periods as short as half an hour

Selkurt Actually it takes an hour or two to impair them seriously, with recovery taking several weeks

Fremont Smith This is a little fast as if these kidneys might be vulnerable to some other factor

Shorr Are these older people?

DeBakey Yes in the neighborhood of 50 to 60 years of age

Shorr They may have started out with reduced kidney reserves

Nickerson In evaluating the vulnerability of different organs, it is important to keep in mind the variable reliability of our tests for dysfunction For example, we have quite good tests to detect changes in kidney function, but we have no way of determining any but the grossest changes in the intestinal tract

DeBakey You are certainly right about that The only test is a purely clinical one in the sense that these patients will have what we call ileus for a period of anywhere from 4 to 6 days Then bowel sounds return and the gastrointestinal function seems to return The patients are then able to tolerate food and to have bowel movements

Dobson There is a great tendency at least clinically to evaluate circulatory function by means of blood pressure because this is the most commonly measured parameter except for pulse rate When questioned, few will admit that they were thinking of pressure as indicative of flow but nevertheless subconsciously most people do think about it as flow This concept can be seriously misleading as in the case of burns where normal blood pressures may go hand in hand with blood flows (cardiac outputs) as low as 15 per cent of normal Thus normal blood pressure cannot be taken to indicate in any way that a burned patient is not in severe circulatory distress or shock The dog at least, is in severe circulatory trouble within 3 minutes after a burn yet his blood pressure may stay perfectly normal for many hours

Rappaport I understand that removal of the section of the aorta was done for aneurysm.

DeBakey Yes

Rappaport What amount of collateral circulation reached these organs during the 2 hours of the operation? This may have been the most important factor With the slow obstruction of the normal blood supply by the aneurysm there probably developed a collateral circulation to the liver and gut When you clamped off the aorta, the pressure above the clamp was raised and more blood was supplied to the collateral vessels

DeBakey That is a very definite possibility and we pointed out that possibility in a previous publication (10) Such collateral circulation is very difficult to measure in the human All we know is that when we exposed the vessels there was a healthy flow through them in the normal direction. It was interesting however to observe that when we had restored continuity at the end of the operation, there was a small degree of retrograde flow from the celiac axis, which indicated that there was some collateral flow to the organs supplied by this artery However there was no retrograde flow from the kidneys

Rappaport In the changeover to the graft, did you still see some pulsation in the distal stumps of the transsected vessels?

DeBakey No no pulsatile flow

Dobson What possibility is there of getting retrograde flow back through the liver from the vena cava? You can certainly perfuse the organ this way I wonder if that is not a possible mechanism when the pressure in the splanchnic area is almost completely eliminated

DeBakey I should think it an unlikely amount

Selkurt It must be mighty low

DeBakey The pressure is very low

Selkurt In the inferior vena cava it is only a couple mm Hg

Dobson Perhaps it does not take very much

DeBakey However if there were a considerable number of adhesions to the spleen there might be considerable collateral flow to the spleen and therefore to the portal vein.

Dobson When a large vein in the abdominal cavity is severed or opened up there is essentially no pressure operating against the hepatic vein pressure There is not very much pressure pushing blood through the liver in the first place since the portal vein and hepatic vein pressures differ very little

Selkurt In the dog the variation is about 7 to 8 mm. Hg

Dobson And in the hepatic vein?

Selkurt It is 2 mm Hg or 3 mm Hg

Dobson If the portal pressure were lowered to zero the reverse pressure gradient would amount to 30 per cent of the normal forward gradient pressure. Suppose this induced a retrograde flow amounting to 30 per cent of normal flow that would be quite an amount of liver flow It is more than is obtained in a shocked animal

Rappaport In the last few years we have been interested in the effect of ischemia on the liver * a central problem also in hemorrhagic shock The ischemia we have been producing is much more severe than that seen after acute loss of blood and is associated with parenchymal damage

In our preparation we perform an Eck fistula on dogs Then 30 hours later we ligate the common hepatic artery (Figure 52) In this way we produce an acute hemorrhagic necrosis for the purpose of bringing about experimentally the phenomena of acute hepatic coma.

Selkurt Isn t there a branch that runs from the common hepatic artery to the pancreas duodenum, and lower stomach?

Rappaport Yes we ligate only the common hepatic artery We intentionally leave the other branches intact. During the 30-hour period after the Eck fistula formation, the common hepatic artery and all its branches dilate, because of the increase of arterial flow to the liver After ligation of the common hepatic artery at the end of the above period, the dilated arterial branches become potential collateral pathways which may increase in diameter later on In a certain per centage of dogs the hepatic necrosis that follows the ligation of the hepatic artery regresses and, at the same time, the clinical phenomena of coma that develop with the acute necrosis disappear (11)

Depriving the liver of blood has its counterpart in pathologic condi tions in human subjects quite frequently in the cirrhotic patient the onset of coma is observed after severe gastrointestinal bleeding At autopsy acute necrosis in the liver is found A recent study from the Mayo Clinic by Dr H Butt and associates (12) is very interesting In the great majority of their fifty two patients that died of acute hepatic coma *recent* necrosis and ischemia of the liver were found at autopsy

Like other investigators of the therapeutic role of increased blood flow to the liver in hemorrhagic shock we were interested to see whether an increase of the hepatic circulation could modify the func tion of a liver severely damaged by ischemia Vessels ligated for about 18 to 48 hours can no longer supply the blood after removal of their

*This work has been supported by Grant 323 of the Defence Research Board Project D50-93-10-31

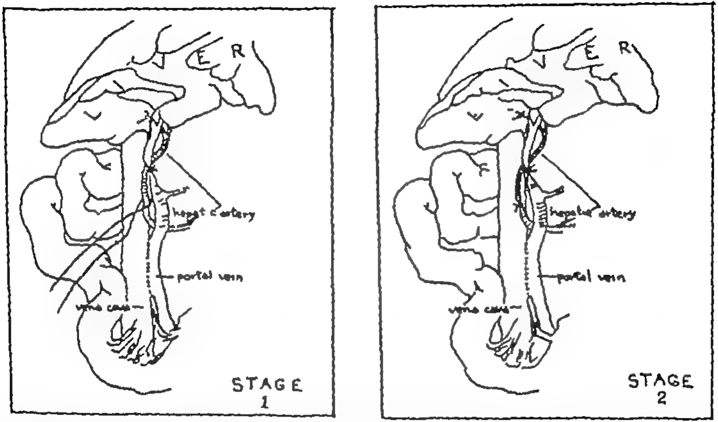


FIGURE 52 Left (Stage 1) Portacaval anastomosis and ligation of the portal stem (Eck fistula) A thread is looped around the common hepatic artery but not tied Right (Stage 2) The common hepatic artery is ligated in the Eck fistula animal Reprinted, by permission, from Rappaport A. M., Macdonald, M. H., and Borowy Z. J Hepatic coma following ischemia of the liver *Surg Gynec & Obst* 97 718 (1953)

ligatures We thought on the other hand that cross-circulation of the ischemic liver with a normal dog would supply not only the blood but also the activity of the normal liver would take care of both dogs the donor and the recipient We turned, therefore to the perfusion of the necrotic liver *in situ* with blood from the dog s own vascular system A strip of motion picture was made to illustrate the method of autoperfusion of the liver

In the dog shown in the film, an Eck fistula had been established 24 hours previously A polyethylene tube filled with heparin was now introduced into the stem of the portal vein above its ligature, *i e*, above the portacaval anastomosis the cannula lay in the portal stem and pointed toward the liver The inferior cava was shown running toward the undersurface of the liver the ligated common hepatic artery was also shown. The polyethylene cannula was passed through the mesentery of the duodenum, a free mesentery in the dog through the epiploon, and was brought out through a stab wound of the abdominal wall The cannula was loose in the abdominal cavity so as not to constrict any intestinal loops

It was fastened to the abdominal wall and remained so for 18 hours During this time a widespread hemorrhagic necrosis of the liver developed. Then 18 hours later the cannula was connected to an extracorporeal circuit that was driven by a small pump with positive action valves The extracorporeal circuit started in the femoral artery passed through the small pump and was connected via the portal cannula to the portal vein

The left femoral artery was cannulated and connected to a manometer for a continuous recording of the blood pressure The motor was switched on and blood was driven in a regulated pulsatile flow into the portal cannula and the liver The arterial autoperfusion was carried out on the anesthetized dog Sometimes instead of perfusing the liver with arterial blood we perfused it with blood from the inferior vena cava via the cannulated femoral vein and iliac vein.

The general view of the experiment showed open ether administration and continuous glucose infusion to replace the missing function of glucose formation of the liver The glucose solution dripped into the jugular vein.

Selkurt Do you have an idea of the volume flow per minute in this system?

Rappaport Yes we know exactly It was possible to see the continuous blood pressure recording and the small pump which was simple and easy to sterilize. The latter consisted of a straight tube that was compressed by the jaws. When the two metal jaws compressed the

tube, the upper valve opened and blood was propelled into the cannula When the jaws released and the tube widened to its normal diameter blood was drawn from the femoral artery into the chamber By setting the jaws we could accommodate more or less blood by using a larger tube, we could have a greater stroke volume. We usually perfused the animal s liver with a volume flow ranging between 70 and 80 ml /min This is because a 10-kg dog has about 250 gm of liver 50 per cent or two-thirds of it had been destroyed by necrosis so only one-third could benefit With this amount of perfusion we were completely satisfied and avoided upsetting the circulatory dynamics

The fact that there were lesions in liver and kidney before perfusion (Figure 53) might be interesting to this group We saw in the autopsy specimens a severe congestion which may indicate faulty inactivation of V D M. The large engorged capillaries are evident in the corticomedullary zone of the kidney (Figure 54) and in the lung (Figure 55) Even when there was a drop in blood pressure at the start of the perfusion (partial embolism) the pump was still able to

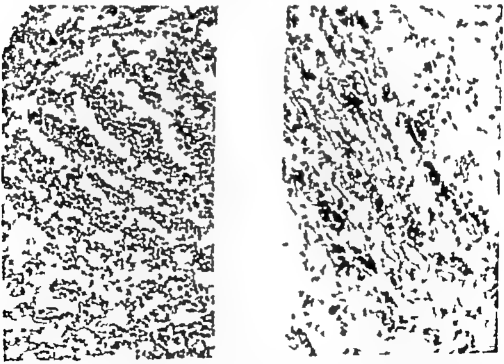


FIGURE 53 Severe congestion of liver and kidney in a dog with acute hepatic failure The hepatic sinusoids (left) are engorged with erythrocytes. The capillaries of the renal medulla (right) are widened and filled with erythrocytes (hematoxylin-eosin, x180)

FIGURE 54 Severe congestion of the renal cortico-medullary zone in a dog with acute hepatic failure. The vasa recta are engorged with blood (hematoxylin eosin, x20, inset x500)

maintain the blood pressure in such a severely ill animal for 6 hours and at the end of the perfusion the pressure was still about 110 mm Hg (Figure 56)

Shorr What was the blood pressure before the pump was put into action?

Rappaport Usually in our dogs it is 120 mm Hg

Shorr And after the liver damage?

Rappaport Around 120 mm Hg in some cases between 110 mm Hg and 120 mm Hg

All the animals that were anesthetized with sodium pentothal required additional dosages of the drug While the injection of half the anesthetic dose (15 mg/kg body weight) gives an unduly prolonged anesthesia and often may kill a dog suffering from acute ischemic necrosis in the dogs that were autoperfused, additional doses of barbiturates had to be given regularly a total range of from 30 to 300 mg during the 6 hours of autoperfusion We therefore decided to investigate autoperfusion

With Dr Hiraki and Dr Rosenfeld we determined the plasma levels of sodium pentothal by the method of Jailer and Goldbaum (13)

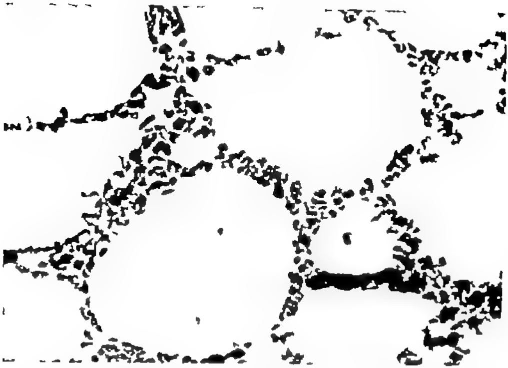


FIGURE 55 Severe congestion of lung in a dog that died of acute liver failure The alveolar septa are splinted with erythrocytes (hematoxylin-eosin, x300)

In a previous series we had injected 15 mg/kg body weight and had determined the plasma levels at 5 and 10 minutes and at 1 2 3 4 5 6 and 24 hours In the normal dog the pentothal level was dropped considerably at 3 hours in the dogs with insufficiency of the liver the level is significantly higher (Figure 57)

To facilitate the comparison of plasma sodium pentothal levels in the groups of animals the plasma concentrations of the drug were expressed as the per cent of the initial value taking as 100 per cent the plasma level 5 minutes after the injection

In our experiments with the autoperfused dogs we decided to watch the 3 hour value because of its significance in the previous investigations As Table LII shows normal nonperfused dogs injected with thiopental sodium, 30 mg/kg body weight had on the average 42 per cent of the initial value at 3 hours When perfused with arterial blood the plasma level dropped to 29 per cent An Eck fistula dog had at 3 hours, 42 per cent of the initial value After perfusion it dropped to 26 per cent Dogs with an Eck fistula and ligation of the common hepatic artery in two stages and not perfused had 48 per cent at 3 hours After perfusion with arterial blood the level 36 is higher than after perfusion with venous blood 32 per cent (Table LII)

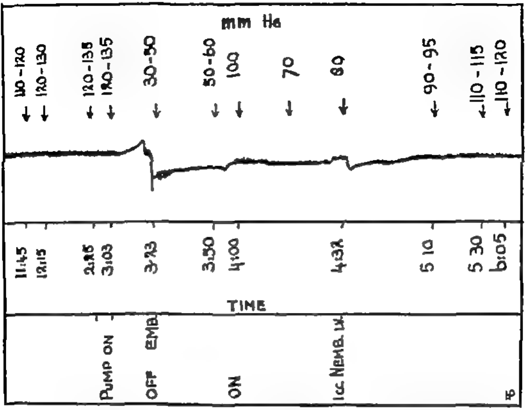


FIGURE 56 Autoperfusion of the liver recording of blood pressure during 6 hours. In spite of the drop in pressure caused by partial embolization of the liver the pressure, 1½ hours after resumption of the autoperfusion had risen to about 110 mm. Hg

From the percentage differences it is evident that there is a definite increase in pentothal detoxication in all dogs in which the hepatic blood flow has been augmented by autoperfusion This effect may be caused by the fact that more pentothal is delivered per minute to the liver for detoxication and hence more is detoxicated It is also possible that the surviving islets of the hepatic parenchyma around the terminal afferent vessels *i e* the cores of the hepatic acini improve their function with the better blood supply

Figure 58 shows the portal field with the core of the surviving acinus and two central veins both at the periphery of the acinus The cores correspond to the circulatory Zone 1 in the hepatic acinus and are the sites where the hepatic arterioles empty into the sinusoids

Figure 59 shows how the circulatory zones are arranged concentrically around the terminal branch of the afferent vessel, and not around the central vein or the portal field While Zone 1 has the best chance to get blood rich in oxygen and nutrients Zone 2 has fewer chances than Zone 1 and Zone 3 is disadvantaged not only with regard to its parent

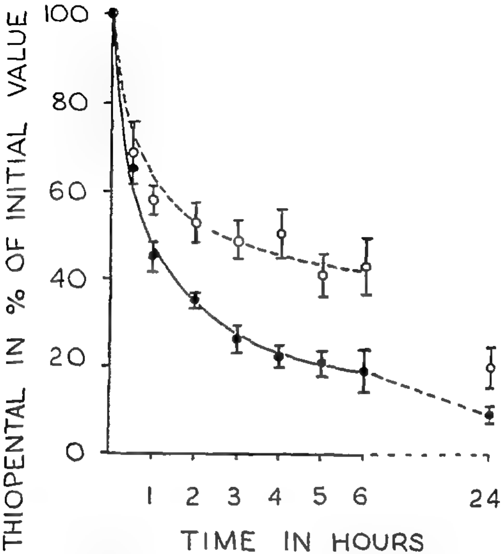


FIGURE 57 Thiopental sodium levels in the blood of seven normal dogs (closed circles) and eight dogs with liver insufficiency (open circles) after the injection of 15 mg of thiopental sodium / kg of body weight. Vertical bars indicate standard errors of the mean Reprinted by permission, from Rappaport, A M., Hiraki, G Y., Rosenfeld, H Cowan, C. R., and Lang, J Effect of autoperfusion of the liver on detoxication of thiopental sodium. *Am J Physiol* 186, *193 (1956)*

vessel but also with regard to the afferent vessel of the adjacent acinus which is a possible collateral *It is as distant from the one as from the other* Therefore the damage that is usually described as pericentral is in fact not pericentral As we have seen in Figure 59 it outlines an area that starts from the portal field extends to one central vein turns

TABLE LII

Average 3 Hour Plasma Sodium Pentothal Levels Expressed As Per Cent of 5-Minute Levels

		Nonperfused (per cent)	Perfused (per cent)	
			Arterial	Venous
Normal	Av (No)	42 (4)	29 (4)	
	Range	(28 to 59)	(19 to 38)	
Eck fistula (EF)	Av (No)	42 (3)	26 (4)	
	Range	(17 to 79)	(13 to 39)	
EF + Lig Common Hepatic Artery	Av (No)	48 (3)	36 (7)	32.5 (4)
	Range	(35 to 64)	(25 to 52)	(17 to 47)

around to the other central vein, and comes back to the initial portal field. It represents the outer limit of the cross section of an acinar unit

The hexagonal field shown in Figure 60 is composed of several parts of such acinar units and in such a section might be found not only parts of acini, the axes of which are in the plane of the section but also tips of acini that lie above or below this section. So it is important to realize that what is seen in a hexagonal field is far from being a uniform mass It is just an association of different parts of units belonging to different acini that lie all either in the field of the section or above or below Attention should always be focused on the afferent vessels The areas around them are important as the sites of entry of nutrients and oxygen they are also the region of the radicles of the biliary system. Here too, ascending infections and all the troubles of the

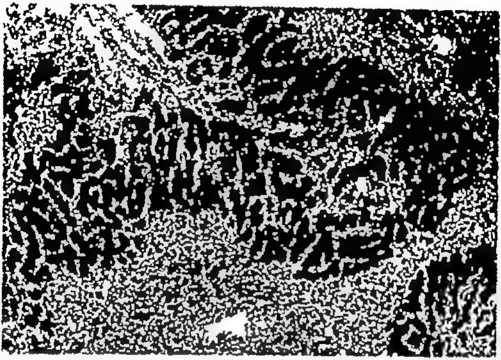


FIGURE 58. Ischemic necrosis of a dog's liver induced by ligation of the common hepatic artery 30 hours after the formation of an Eck fistula. The axial afferent vessels of the surviving acinar core branch out from a small portal field (left upper corner). The necrotic tissue occupies Zone 3 and part of Zone 2 of an hepatic acinus. It begins around the central vein and breaks through the tip of Zone 3 into the adjacent hexagonal field where it joins the pericentral necrotic area. The central vein of adjacent hexagonal fields are thus linked by a band of necrotic tissue (Wilson's ORO stain x55)

biliary obstruction will first become apparent. This is the dynamic zone the activity of which should be kept in mind.

Figure 61 shows an hepatic sinus in a human liver. The liver was injected with India ink, and cut just parallel to the terminal branch of the portal vein. The acinal structure can be seen extending from the periphery of the acinus of one central vein to that of the other central vein.

Each acinus is in fact part of a more complex structure (Figure 62). We define the complex acinus (14) as the amount of tissue that is arranged around a trio of *preterminal* channels which divide into their terminal branches indicated in this figure by 1—4. All the small clumps of parenchyma each of them around a trio of *terminal* channels form together the complex acinus. In Figure 62 the unity of parenchyma was disrupted by a mild ischemic necrosis induced at the circulatory periphery of the acini. India ink was injected through the

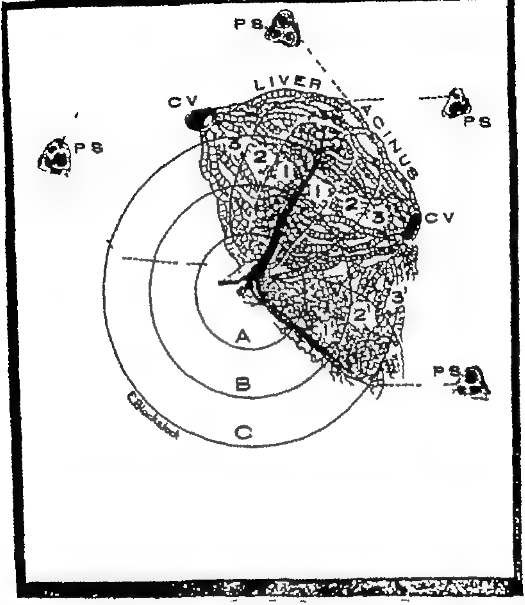


FIGURE 59 Circulatory zones in the hepatic acinus. The liver acinus occupies adjacent sectors of neighboring hexagonal fields P.S. = portal space C.V = central vein Zones 1 2, and 3 respectively represent areas supplied with blood of first, second, and third quality with regard to oxygen and nutrients These zones center about the terminal afferent vascular branches and extend into the portal field from which these branches originate Zones 1 2 and 3 designate corresponding areas in a portion of an adjacent acinar unit In Zone 1 and 1 the afferent vascular twigs empty into the sinusoids. The circles A B, and C delimit concentric bands of hepatic parenchyma around a small portal field They mark the circulatory zones in decreasing order of nutrients and oxygen content as assumed in the classical concept of hexagonal lobules. Reprinted, by permission, from Rappaport, A. M., Borowy Z. J Lougheed W M., and Lotto W N Subdivision of hexagonal liver lobules into a structural and functional unit role in hepatic physiology and pathology *Anat Rec* 119 *11 (1954)*

FIGURE 60 The human liver acini in the hexagonal field Irregular clumps of tissue (acini) in one hexagonal field with central vein (C.V.) in its center The axial vessels branch out from three small portal spaces (PS_1 PS_2, PS_3) in the corners of an hexagonal field The cut tip of an acinus is also visible above the central vein (India ink injected, 100 μ thick, cleared section x110)

portal vein in order to outline the terminal afferent vessels and sinusoids of the surviving parenchymal clumps These are represented as if they had been peeled out of their necrotic outskirts. The amount of necrosis induced can be judged by the three shelves the remnants of necrotic tissue that surrounded the central veins and involved the circulatory periphery of the acini

But we can go higher as in the human liver shown in Figure 63 This is injected with India ink through the portal vein and it is found that the complex acinus is a part of an acinar agglomerate A preterminal branch is shown the clump of tissue around it representing a complex acinus while the whole structure is an acinar agglomerate Slight damage might appear first around the entire structure but later when the injury has increased it will appear at the circulatory periphery of the smaller structural units forming the agglomerate

Figure 64 shows a dog's liver with induced severe ischemia Ischemic necrosis evolving in this field has broken down the tissue into its acinar

FIGURE 61 Human liver acinus. The acinus occupies sectors only of two adjacent hexagonal fields and reaches their central veins (C.V.) The axial terminal portal branch of the structural unit is injected with India ink and runs perpendicularly to the two hepatic (central) veins with which it interdigitates. It is shown by a cut parallel with almost its entire length. The central veins lie close to each other in this section (thick cleared section x300) Reprinted by permission, from Rappaport, A. M. Anatomic considerations. *Diseases of the liver* L. Schiff Editor Philadelphia, J B Lippincott Co., 1956.

constituents The large branch and the parenchyma arranged around it and its branches constitute an acinar agglomerate.

Each acinar agglomerate is part of another agglomerate of higher order and a number of these may form a small liver lobe in animals with a multilobular liver In the human liver the order of the agglomerates follows closely the magnitude of the efferent biliary and afferent vascular branches upon which they are oriented

Figure 65 is a semi schematic drawing showing the hepatic vascular architecture We have represented this piece of liver as if we had cut out a cuneiform part to demonstrate the longitudinal aspect of a small portal space with its component structures For simplification only terminal portal venules branching out from the portal vein in this space are drawn The blood coming up in the portal and arterial vessels supplies the hepatic units of several hexagonal fields The arcuate course of the terminate vessels which form the axes of the irregularly

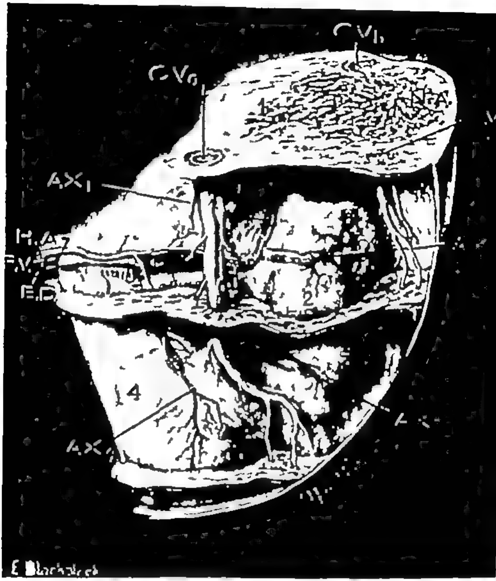


FIGURE 62 Complex liver acinus. Tridimensional reconstruction after 150 serial sections, each 5μ thick This complex acinus lies beneath the hepatic capsule. H A. P V., and B D = hepatic artery portal vein and bile duct forming together the axis of the complex acinus and running perpendicular to the central veins (CV CV$_b$, and CV) AX_1 AX_2 AX_3, and AX_4 = the trios of terminal branches of H.A., P V., and B.D each of them forming the axis of a corresponding simple acinus (1 2 3 4) AX_4 and AX_3 course perpendicular to CV AX_2 lies perpendicular to CV and CV AX_1 runs obliquely to CV and CV_b. N A = section of a neighboring acinus growing down from above and lying beneath the hepatic capsule Most of the India ink seen in the plane of section stems from the injected axial vessel of N A There is no axial vessel visible in the sectioned tip of acinus 1 Reprinted, by permission, from Rappaport A M Anatomic considerations. *Diseases of the Liver* L Schiff Editor Philadelphia J B Lippincott Co., 1956.

FIGURE 63 Acinar agglomerate in human liver injected with India ink Three large portal branches grow out in different directions from a portal space (P.S. in right upper corner) One of these runs diagonally through the field and represents the axis of an acinar agglomerate. From this portal branch preterminal (1) and terminal (2) branches grow out and form the axes of the outcropping complex and simple acini respectively (thick 100 μ cleared section x18) Reprinted, by permission, from Rappaport, A. M. Anatomic considerations. *Diseases of the Liver* L. Schiff Editor Philadelphia, J B Lippincott Co 1956

arranged structural units is shown There are many anastomotic pathways permitting the flow of blood in all directions and also shown are the many collateral connections between the acinar structures that allow flow of blood in all directions and that create the uniformity of the parenchymal mass, which is not in itself uniform Cuts are shown parallel to terminal vessels and the acinar structures around them are shown in cross section

In Figure 65 are shown also the circulatory Zones 1 2 and 3 which are very helpful in the orientation of necrotic, cirrhotic, and fatty lesions It will be found that the distribution of all these lesions follows the arrangement of these zones rather than the conventional ones (periportal and pericentral)

Figure 66 shows another liver injected with India ink. A central vein and the lower part of a hexagonal field are shown In one area it is evident that there is no uniform parenchymal mass while in another

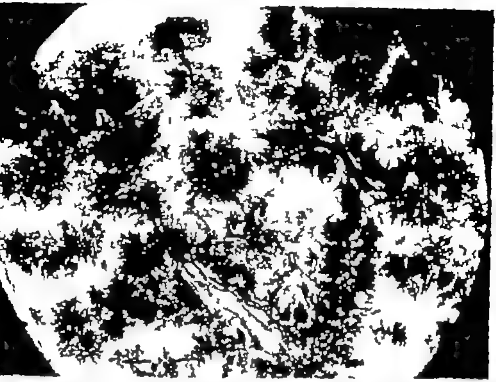


FIGURE 64 Acinar agglomerates outlined by ischemic necrosis in a dog's liver Necrosis was induced by simultaneous formation of an Eck fistula and ligation of the common hepatic artery India ink was injected into the cannulated portal stem filling the large vessels of the acinar agglomerates, the axial vessels of the complex, and simple acini and their sinusoids. The ink does not enter the necrotic areas. Only the groups of acini close to the large afferent vessels have survived (Thick 100 μ cleared section x18)

part an acinus has been cut rather close and parallel to its axial structures that is the reason why it seems so well filled with the ink. In another part of the field another acinus extends from one central vein to the other but it is less filled with ink because the cut is closer to the acinar periphery It is an acinus the axis of which lies above or below the plane of the section The diversity of the ink in the different parenchymal clumps proves that the tissue around the central vein is far from being one unit

After this diversion into the acinar concept of hepatic structure we return to the autoperfusion of the liver We were interested to see whether the effect of autoperfusion is caused only by a greater amount of sodium pentothal brought into the liver or whether there were some histochemically detectable improvements in the islets of tissue that survived hepatic ischemia The histochemical study of the tissues was done by Dr W G B Casselman who is an Assistant Professor in

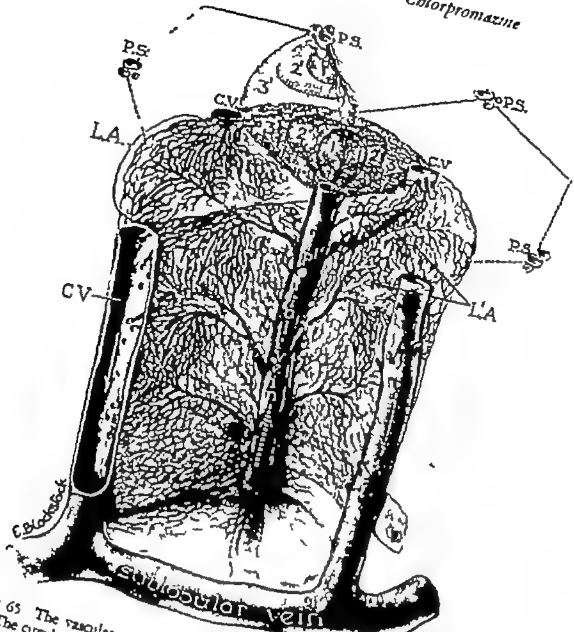


FIGURE 65 The vascular architecture of the liver in its relation to the acinar units The circulatory zones in the center acinus are marked as 1 2 3 those in an adjacent acinus as 1′ 2′ 3′ L.A. = hepatic acinus L.A = hepatic acinus penetrating an hexagonal field which is situated well above the level of its origin P.S. = small portal space and C.V = central vein. Reprinted, by permission, from Rappaport, A. M. Borowy Z. J., Lougheed, W M and Lotto W N Subdivision of hexagonal liver lobules into a structural and functional unit role in hepatic physiology and pathology *Anat Rec* 119 11 (1954)

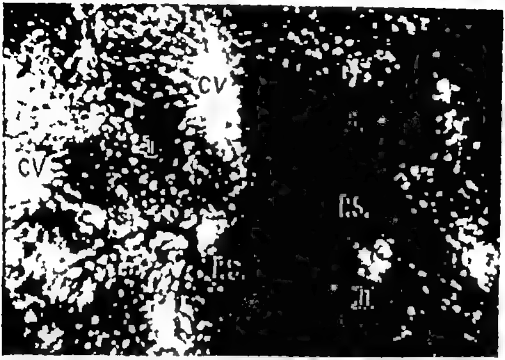


FIGURE 66. The acinar units and the hexagonal field The tissue around a central vein (C.V., near center of upper half of the field) is composed of parts of acini (e.g. I II and III) which form the lower half of a hexagonal field. Their ink filled axial vessels grow out from two small portal spaces (P.S., P.S.) The tissue of the upper part of this field stems from other acinar units. Acinus I is seen to be drained by two "central veins (C.V C.V) (Human liver injected with India Ink cleared thick section x110) Reprinted by permission, from Rappaport, A. M. Anatomic considerations. *Diseases of the Liver* L. Schiff Editor Philadelphia, J B Lippincott Co. 1956.

the Banting and Best Department of Medical Research of the University of Toronto Toronto Canada. I will present first the normal liver autoperfused for 6 hours There are no changes in it So we must say autoperfusion in itself does not damage the liver

Figure 67 is the liver of a nonperfused animal The amount of necrosis present in the liver is apparent (Figure 68) Only small remnants of tissue around the afferent vessels have survived

Figure 69 is a higher magnification that shows a branching terminal vessel A small ring two or three cells wide of surviving tissue cells filled with nonacidic fat is shown

Figure 70 is also a liver of a nonperfused animal but in another section and it shows that fatty change precedes the necrosis of the cells which are heavily infiltrated with fat

When a necrotic liver is autoperfused for 6 hours with arterial

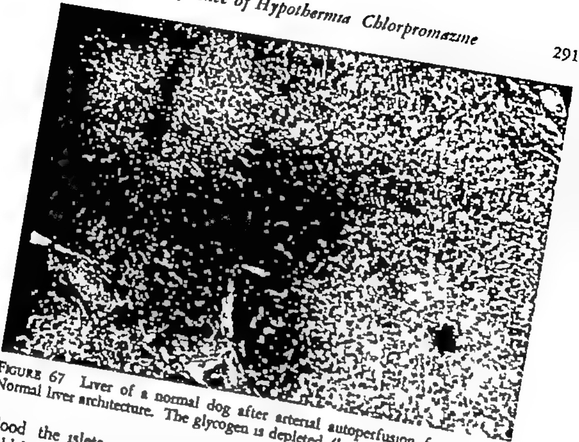


FIGURE 67 Liver of a normal dog after arterial autoperfusion for 6 hours. Normal liver architecture. The glycogen is depleted (hematoxylin-eosin, x110)

blood the islets around afferent vessels coming out from the portal field look much better There is very little fat in the parenchymal rim of tissue, shown in Figure 71 and its outer row of cells Of course there is fat in the necrotic area.

When such a liver is perfused with venous blood the islets do not look bad, but in some parts the outer margin is still nearly filled with fat (Figure 72)

In Figure 73 there is something of interest for this conference This is the liver of an animal that died on the table after 4½ hours of hemorrhagic shock A portal field can be seen and the terminal vessels branching out of it. The cells around the vessels would correspond to Zone 1 and Zone 2 Central veins can be seen as well as a heavy dusting of fat in the outer Zone 3

In conclusion, we must say that arterial autoperfusion of the liver speeds the detoxication of thiobarbiturates in the liver (*a*) by delivering to the liver for detoxication a greater quantity of the drug than is offered in ischemia and (*b*) possibly by improving the condition of the parenchymal cells that have survived the liver damage

Thus it may be suggested that when cases of pentothal poisoning do not respond to the usual therapy autoperfusion of the liver

FIGURE 68 Liver of an Eck fistula dog with subsequently ligated common hepatic artery Massive hemorrhagic necrosis 24 hours after ligation of the hepatic artery Only narrow rims of cells around portal spaces survive. The cells in the outer row adjacent to the necrotic areas, are filled with fat droplets (Wilson s ORO stain, x110)

with arterial or venous blood should be attempted Our findings may also help those carrying out experiments on hemorrhagic shock in which pentothal anesthesia is used

DeBakey Does it make any difference whether arterial or venous blood is used?

Rappaport In terms of the percentages of sodium pentothal the differences are not very great

DeBakey I mean in the effect

Fremont Smith For recovery venous blood is about as good as arterial?

Rappaport It seems so

Selkurt Where do you tap in to get the blood?

Rappaport This is the preparation with intrahepatic circulation curtailed by an Eck fistula and partial ligation of the arterial supply We tap the femoral vein to get the blood for autoperfusion from the inferior vena cava

DeBakey You are [illegible] ed through the other source aren t you?

FIGURE 69 Tangential cut of axial vessels. The cells in the outer row of the narrow rim of intact parenchyma surrounding the terminal vessels are filled with fat (black) Fat droplets are visible in the adjacent necrotic areas (Wilson's ORO stain, x18)

Rappaport Yes If *all* branches of the hepatic artery were ligated in an Eck fistula dog, it would be another preparation However such an animal does not survive 24 hours, even when supplied with antibiotics.

DeBakey Even when you autotransfuse?

Rappaport I do not autotransfuse. The animal dies before that

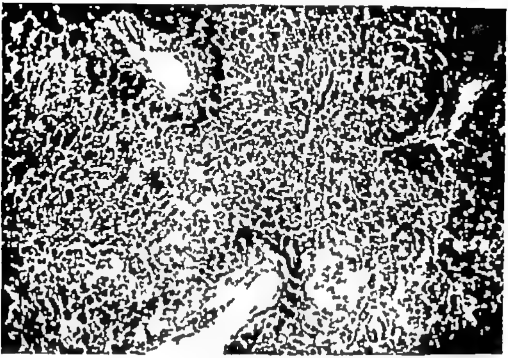
Shorr These animals are not pretreated with an antibiotic?

Rappaport They *are* treated with antibiotic.

DeBakey They will die if you do not have the Eck fistula?

Rappaport If I ligate the portal vein and do not make an Eck fistula, such a dog dies within half an hour because the portal vein in the dog does not have sufficient collaterals. After ligation of the portal vein, the portal bed is engorged with blood and the animal dies on the table If a dog dies 2 to 3 hours after the formation of an Eck fistula it can be predicted that a clot obstructing the lumen of the fistula will be found at autopsy

Shorr You might be interested in the study Dr Baez did a few years ago, in which he arterialized the liver without, of course, con-



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DeBakey You are getting some blood through the other source aren't you?

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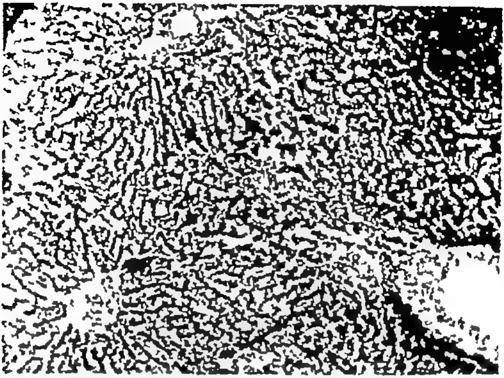

FIGURE 70 Ischemic nonperfused liver of a dog 55 hours after the formation of an Eck fistula and 27 hours after ligation of the common hepatic artery Necrosis is starting around the central veins (left upper and lower margin) The remainder of the parenchyma shows the severe fatty change that precedes the cell necrosis (Wilson's ORO stain, x110)

trolling blood pressure or flow Would you discuss those studies Dr Baez?

Baez. These experiments were designed to see whether animals whose liver received only arterial blood would be more resistant to our standard hemorrhagic shock procedure. First under pentobarbital anesthesia, the right kidney was excised saving as much of the renal artery and vein as possible Then the portal vein was freed of perivascular tissue and sectioned between two clamps Within 7 to 10 minutes an Eck fistula was established by end to-end anastomosis of the peripheral stump of the portal vein and the renal vein. The renal artery was then anastomosed to the hepatic (central) stump of the portal vein by means of an arterial graft 1 to 1½ inches in length depending on the length of the renal artery (Figure 74)

Dogs successfully operated upon recovered fully and appeared to be perfectly normal and healthy Hemorrhage was induced 2 to 21 months after operation As already reported (15 16) these dogs required greater blood loss than their controls to achieve the same

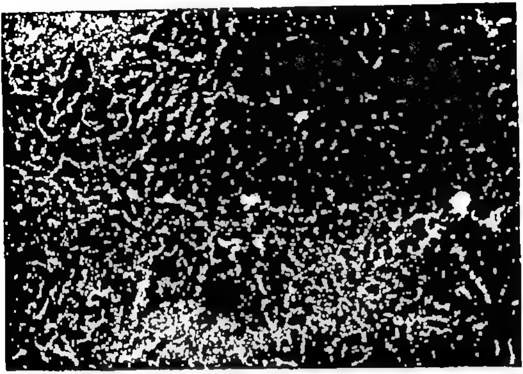

FIGURE 71 Ischemic liver of a dog autoperfused with arterial blood for 6 hours. Ischemia was induced by the formation of an Eck fistula and ligation of the common hepatic artery 48 hours and 19 hours, respectively before perfusion. A band of necrotic tissue can be seen stretching across the center of the field it contains fat droplets. The surviving islets of parenchyma above and below this zone of necrosis are well preserved and contain little fat even in the cells adjacent to the necrotic area. Portal field in left upper corner (Wilson's ORO stain, x110)

degree of hypotension, they had only mild V.D M. activity in their blood, and compensatory behavior persisted in the terminal vascular bed for a longer time than in the controls Plasma uric acid values did not increase as much as in control dogs subjected to the same shock procedure A number of these dogs died from accidental hemorrhages after the experiment, so our series was small (Table LIII) However our impression was that all of them would have been reversible, in contrast to the high mortality in their controls All survived We have six now

Rappaport I should expect that.

Fremont Smith What about the livers? Did you do liver slices?

Baez No we did not do any in this series of dogs

Fremont Smith In other words, they act as if they had been given aureomycin beforehand?

Shorr As if they had been given arterial blood at arterial pressure.

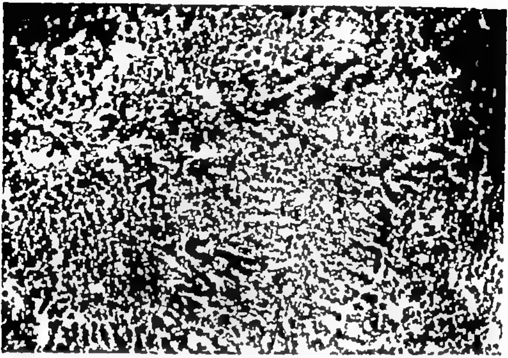

FIGURE 72. Ischemic liver of a dog autoperfused with venous blood for 6 hours. Ischemia was induced by the formation of an Eck fistula and ligation of the common hepatic artery 49 hours and 20 hours, respectively before the perfusion. Necrosis has developed at the acinar periphery where the fat laden (black) cellular debris are seen. The cells at the margin of the surviving acinar core show less fatty change than in the unperfused livers (Wilson's ORO stain, x110)

We do not think the volume of the blood is as important as its arterial character and pressure.

DeBakey If I understood this correctly all of the portal blood flow was returned to the vena cava you short-circuited the liver

Short By diverting the portal blood into the renal vein.

DeBakey And the proximal stump of the portal vein was anastomosed via a graft to the main artery so you had a double arterial supply

Baez Yes

Tremont Smith Both through the portal veins and hepatic artery

Rappaport There was some previous discussion on a similar topic I mentioned that by microscopic examination *in vivo* it was found that in hemorrhagic shock the liver parenchyma is almost exclusively supplied via the arterioles and that the oxygen saturation in the portal vein drops tremendously during hemorrhagic shock Blood is diverted to the extrahepatic tissue and the oxygen is taken out there. So that in hemorrhagic shock the liver survives the whole time on arterial blood A preparation with double arterial supply to its liver *i e.*, with

FIGURE 73 Liver of a dog that died after 4½ hours of hemorrhagic shock. The heavy dusting of fat (black) is seen in Zone 3 which circles around the axial vessels that branch out from the portal field (left lower corner) and run diagonally toward the right upper corner The cells in Zone 1 close to the axial structures, contain least fat. The central veins are not included in this field (Wilson s ORO stain, x50)

its normal hepatic artery and with the renal artery emptying into the portal vein, should do better in hemorrhagic shock.

Shorr Yes exactly Would this preparation be something for you to think about, Dr Engel, in terms of your metabolic studies? You might find something other than the ferritin systems being modified under these circumstances

Engel As I listen to this discussion it seems to me that we are probably closing in on certain things During the discussion, we have heard of certain agents and techniques that have extended life after trauma or bleeding by changing the decrements mentioned before Agents such as chlorpromazine, dibenzyline, aureomycin etc and techniques like refrigeration present, in some respects the equivalent of say what was done years ago by introduction of transfusion They enable the circulation to carry on just a little bit further but in some respects only delay the inevitable Then conversely there are certain deleterious influences such as the bacterial factor Dr Fine mentioned

FIGURE 74 Anastomosis of renal artery to hepatic stump of portal vein and Eck fistula in dog giving the liver an entirely arterial blood supply 1 Aorta. 2 Vena cava. 3 Renal vein. 4 Portal vein (peripheral end) 5 Renal artery 6 Arterial graft. 7 Portal vein (hepatic stump) 8 Liver 9 Pancreas.

This is another insult which when removed, delays things but does not solve them Then there are certain tissue changes, and here the liver still appears to be of central importance. It does seem as though something *is accomplished by protecting the liver whether by improving the* hepatic circulation and preventing hypoxia or by use of agents which can influence the metabolic activity of the liver so as to enable it to *function better under hypoxic conditions*

For the purpose of the discussion at the moment, it does not matter whether one argues that the ferritin mechanism is good bad or indifferent This will not change the basic argument that there is a need *to determine whether or not other metabolic parameters in the liver* are important and can be modified favorably and unfavorably by various agents as has been the case with ferritin Moreover there should *be an investigation of procedures which induce shock by directly influ-* encing the enzyme systems including those that are being studied in the ferritin-inactivation system

We really do see clinically certain types of shock that are caused by agents which act on enzyme systems The most obvious one is arsenic poisoning This is a good sulfhydryl poison, and it is very well known

TABLE LIII

Hemorrhagic Shock in Dogs with Arterialized Livers

Exp No	Duration of Hypotension Total min	Duration of Hypotension ± 35 mm Hg min	Maximal Bl. Loss % body wt	Sustaining Transfusions % body wt	Result
		1 Control Series			
1	260	170	5 1	2 7	died 14hr
2	240	120	5 0	3 0	died 2hr
3	270	150	5 5	1 0	died < 2hr
4	250	155	5 0	1 0	died 24hr
5	250	120	4 9	3 3	died < 2hr
6	240	150	5 0	2 1	died < 3hr
7	280	150	4 9	1 1	died 34hr
Avg	258	145	5 05	2 01	died 12hr
		2 Arterialized Liver			
1	270	150	6 15	0	survived
2	300	180	6 10	0	survived
3	240	185	7 10	0 3	survived
4	270	165	7 10	0	survived
5	315	180	6.4	1 4	survived
Avg	279	175	6 57	0 34	survived

that shock is part of the clinical picture of acute arsenic poisoning

We have not given much thought in the past to what might be learned by using poisons with known metabolic actions to induce shock. This is something that should be studied One poison that we are interested in which may have significance for the shock problem is sodium fluoroacetate We were not specifically concerned with shock when we began to study the metabolic consequences of this agent which acts by blocking the Kreb s cycle However after working with it several years, we have been more and more impressed with the fact that this poison makes rats extraordinarily susceptible to developing shock after really very minor stresses such as handling or taking blood

samples It is interesting too that this does not seem to happen immediately but only 12 to 16 hours after poisoning with sodium fluoroacetate This is when the animals are most susceptible. Here shock develops some hours after animals receive a poison which blocks the Kreb s cycle It could reasonably be assumed that the eventual effect of the poison which blocks the Kreb s cycle would be a running down of oxidative metabolism Thus failure of oxidative metabolism is just what might be anticipated to occur late in shock when irreversibility sets in

One of the next directions for research in shock is attention to the effects on the circulation of agents which directly influence these enzyme systems which we know are disrupted by hypoxia In other words, can irreversible shock be induced readily by poisoning certain enzyme systems and can it be shown that the metabolic disturbances in such organs as the liver precede the decompensation of the circulation and the development of hypoxia? From studies of this sort it might prove possible to establish which of the metabolic derangements are critical and which are purely secondary to hypoxia

Fremont Smith Does cirrhosis of the liver also come into the category?

Engel Clinically we know that patients with cirrhosis of the liver seem to be more susceptible to shock particularly from hemorrhage and infection

Fremont Smith I might just mention at this point that this all started when Dr Shorr who had been interested in Zweifach s and Chambers studies * as reported in our first shock conferences during the war also attended the liver conferences during the war and saw Dr Paul György s nutritionally deficient rats with cirrhosis of the liver * Someone asked Dr György why he did not get serial biopsies to show the development of this cirrhosis He said he could not because the rats would not survive a biopsy Several of us questioned this since it is well known that rats will survive removal of more than half of the liver It was then that Dr Shorr got the idea these rats were already on the edge of shock and very susceptible to trauma

Shorr No this was actually only in relation to cirrhosis We found the nutritionally cirrhotic rats had defects in their hepatic ferritin systems which resembled those in shocked dogs It gave our own studies further impetus

Engel Patients with cirrhosis and bleeding varices usually seem much more difficult to handle than patients with a bleeding ulcer with comparable blood loss

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* Unpublished

DeBakey At the same time, a patient with cirrhosis of the liver can tolerate hepatic artery ligation very well. I suppose this is because in cirrhosis considerable collateral blood flow has already occurred

Rappaport I will not say that. From what some surgeons some times cause, when they ligate the hepatic artery supplying a cirrhotic liver they are repeating the second stage of our experimental procedure for inducing hepatic coma (and they have successfully induced it in quite a number of cases) The first stage the Eck fistula, is produced in the cirrhotic patient by nature. Over the years of the disease the portal blood is diverted through collaterals into the inferior vena cava and the abdomen. So the Eck fistula is formed. When the surgeon opens the abdomen and ligates the hepatic artery he merely brings about the second stage, and he should not be surprised when a certain percentage of patients die These deaths are listed as liver shock, hepatorenal syndrome, and under all kinds of other names but the picture before death is that of pure hepatic coma. I do not deny that some patients survive I know they do

DeBakey The proportion of patients that die following that procedure has been relatively small, as a matter of fact, it is unusual In our own series of cases, now numbering thirteen, in which that was done, not a single death of that kind occurred. Every patient tolerated the operation quite well. The deaths occurred later not in a matter of days or weeks, but months So far as we could determine, the procedure did not affect the clinical course in any way in a high proportion of these patients I mean by that, that we seem not to have had any adverse effect on the patient.

Shorr What about the collateral circulation?

DeBakey I should say that the collateral circulation must have been excellent

Shorr Yes that is the point

Rappaport The cases that survived have, in the process of their disease had the portal blood shunted away and the liver supplied by all kinds of arterial collaterals not only through the hepatic artery but also from the celiac and mesenteric trees If the patient is fortunate enough to have such good collaterals and we know there are many of those listed as aberrant arteries in the human liver that patient is provided by nature to give success to the surgeon.

DeBakey The only point I wanted to make was that I do not think that the procedure, on the basis of our clinical experience with it in thirteen cases, has any therapeutic value. That is why we stopped at thirteen we did not see that it made any difference in the subsequent course of the patient

There were some who survived as long as 3 years Again, we did not notice any change in the course They had recurrent hemorrhages, and their general status was not significantly changed as far as we could tell The interesting thing was that they were able to tolerate the procedure.

I might say that we not only ligated the hepatic artery but we also ligated the splenic artery and the left gastric artery

Rappaport They had good phrenic arteries they must have had good collateral flow from the superior mesenteric artery and probably a lot of collateral circulation coming up with the bile duct as I observed in our animals We were able to produce such a liver preparation in a series of eighteen dogs the so-called paradoxical dogs We diverted totally the portal blood from the liver and ligated all branches of the hepatic artery and the organ survived on collateral circulation alone It lived on what it borrowed from the neighbors (17)

Shorr Dr Furchgott, would you tell us a little about your work on G D 131?

Furchgott G D 131 has no adrenergic blocking activity on rabbit aortic strips in concentrations which are comparable to those used in your experiments or Dr Nickerson s earlier experiments on whole animals On the other hand, it has a very definite potentiating effect on the contractile response of aortic strips to epinephrine. Such a potentiating effect we have not picked up with any concentrations of related β halo alkylamines such as dibenamine or SY 28 which act simply as adrenergic blocking agents

However we have found G D 131 to have one pharmacologic action in common with dibenamine and SY 28 Dr Harvey and Dr Nickerson (18) have already reported on certain common chemical reactions of β-halo alkylamines such as reactions with SH groups The common action which we find is on the isolated left atrium of the guinea pig driven electrically *in vitro* All three of these agents have a stimulating effect on the force of contraction of this isolated cardiac muscle One of them SY 28 we have tried on spontaneously beating atria. Here it has been found to have a stimulating effect on rate as well as force like epinephrine or norepinephrine I predict that the two other agents would do the same *

Thus when we add them to heart muscle these agents seem to act very much like epinephrine or norepinephrine but the duration of their action is limited that is tachyphylaxis occurs For example after the electrically driven auricle at 37°C. has been exposed to one of these agents for 30 minutes or so the stimulatory effect on force begins to

*In more recent experiments, this prediction has proved correct.

disappear After about an hour of exposure, little stimulation is evident and the preparation can be washed, the agent reapplied and there will be no increase in force

Do these agents act directly like epinephrine or norepinephrine, or indirectly? So far, our preliminary evidence indicates that they act indirectly by liberating epinephrine or norepinephrine from the sympathetic fibers which are in this cardiac preparation and that the tachyphylaxis is a result of damage or exhaustion of these fibers on prolonged exposure to these agents

This common action on cardiac force of three β halo alkylamines two of which are adrenergic blocking agents and one of which is not, is interesting from a pharmacologic standpoint, but I do not know whether it throws any light on the action of β halo alkylamines in protecting against irreversible shock.

Shorr It might To be sure that this agent G D 131 which had lost its adrenergic blocking properties but retained the effect on the liver ferritin systems was not an antibiotic, we asked Dr R M. McCune Jr and Dr P Dineen (19) at Cornell to test it against a group of organisms—*Staphylococcus aureus*, a specific streptococcus (Group A) —C-203 *Proteus vulgaris Escherichia coli Salmonella Klebsiella pneumoniae, Clostridium welchii* and *Pseudomonas aeruginosa* They used G D 131 in concentrations of 50 and 250 μg., and found no evidence of any antibiotic activity no inhibition of growth in their tests Aureomycin would be effective at the 50-μg level for all these organisms, and at 3 μg for *Clostridia* So the amount used was well in excess of the effective level for an antibiotic and we can be certain that it is free of that particular property

Hass This is somewhat out of context now but it is another effect of cold In our laboratory Mr Schachter Mr Sidlofsky Mr Hamilton, and Dr Baker found that under some circumstances previous acclimatization to cold had a beneficial effect on the course of development of shock. Rats were acclimatized to cold by keeping them for 6 weeks or longer at 1° to 2 C. They were then taken out of the cold room and put in a constant temperature box at 27 C. and shocked by a clamping technique The clamps were placed on the hindlimbs, left on for a period of 10 hours and then removed The limbs became swollen and shock developed. The animals which were previously acclimatized showed a slower fall in body temperature, a slower fall in metabolic rate and a longer survival time These animals differed from the hypothermic animals mentioned earlier in the discussions in that their body temperatures were normal at the start of the test Figure 75 shows the changes in rectal temperature with time The times of clamp appli

cation and removal are indicated by the vertical lines. The curve S indicates the temperature changes in two cold acclimatized animals that survived The curve A (S) indicates the temperature changes in the cold acclimatized rats leaving out the two that survived The temperature changes in shocked control unacclimatized rats is shown in C. These were age controls for the acclimatized rats but the weight controls gave similar results The *significance of the difference in temperatures* between acclimatized and unacclimatized was tested at 5 hours *after clamp release* At *this point a t-test showed* p to be less than 0 02 Figure 76 shows the percentage of the rats surviving at a given time. Two of the acclimatized animals survived for longer than 48 hours The mean survival times and standard deviations for the different groups are given in the upper right corner A comparison of acclimatized and controls gives p values less than 0 01 Figure 77 shows the changes in oxygen consumption in these animals The dotted vertical lines indicate the period of clamp application The line marked Cold refers to the cold acclimatized rats and that marked Room Temperature refers to the controls The acclimatized rats have a higher

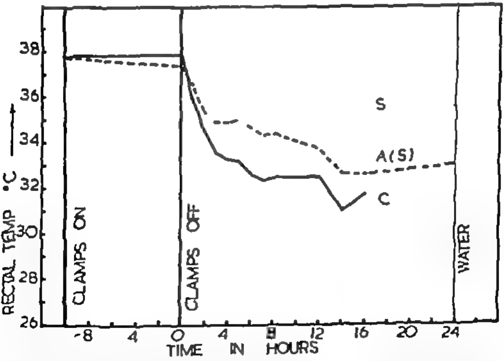

FIGURE 75 The changes in rectal temperature with time S indicates the temperature changes in 2 cold acclimatized rats that survived A(S) shows the temperature changes in the other cold acclimatized rats C indicates the temperature changes in shocked control rats which were not acclimatized.

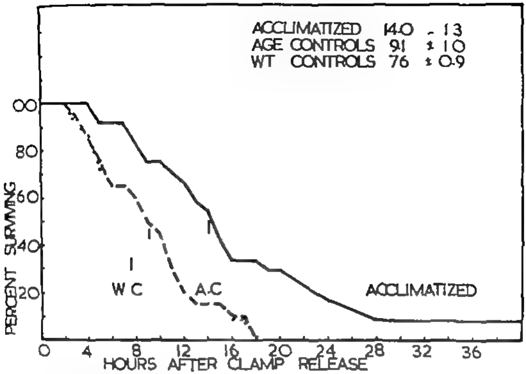

FIGURE 76. *The percentage of the rats surviving at a given time. Mean survival times and standard deviations are shown at the upper right*

A.C. = age controls

W C. = weight controls.

oxygen consumption at the start. During the period of clamping at 27°C. the oxygen consumptions come closer together During the period when shock is developing the ones that had been cold acclimatized showed a slower fall in metabolic rate I do not know the explanation for these results but we thought they might be of some interest The acclimatized animals have a larger blood volume than normal They also have a higher metabolic rate and their adrenals are very large. We may be observing a phenomenon related to that suggested by Dr Levine earlier in the discussion

Shorr Actually they would suggest that (if we disregard the possible effect of the higher blood volume, which of course we cannot) in a sense because their oxidative needs are greater these animals are more jeopardized than the animals which have a lower metabolic rate

Hart You would think so yet they seem to do better

Fremont Smith I do not believe we know anything about the physiologic processes involved in acclimatization to cold do we? Is there

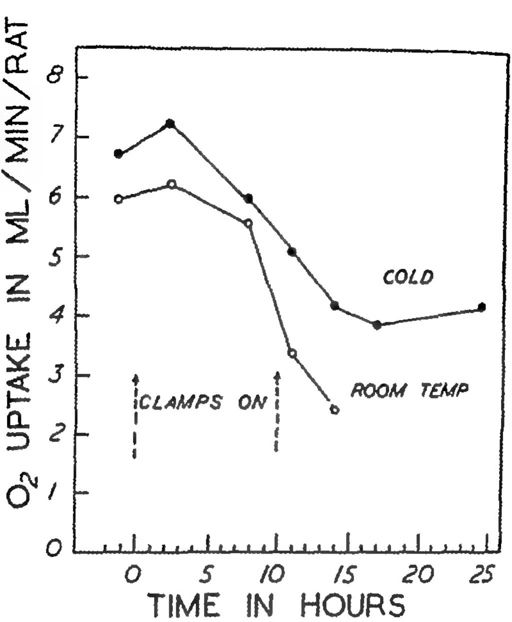

FIGURE 77 Oxygen consumption in cold acclimatized (cold) and control (room temperature) rats.

greater reactivity of the sympathetic system? Or are there any other indications as to how this operates?

Haist I do not know

Fremont Smith This would be an essential clue perhaps as to why they are protected

Haist It is possible that experiments of this type might give leads concerning acclimatization quite apart from any effects on shock

DeBakey Hasn t there been considerable work on high altitude, for example?

Fremont Smith You mean to cold?

DeBakey Not just for cold, but for acclimatization to atmospheric changes as well

Fremont Smith There is definite information on high altitude, but as far as I know there is no information on specific processes to cold acclimatization There is no reason to believe the two studies would be identical although there might be overlaps

DeBakey I was thinking in terms of overlaps It seemed to me there were some studies by the Army group at Fort Knox on acclimatization to cold

Fremont Smith The Army group were here at our conference and I do not believe that anyone brought out any indications as to what was involved.

Hast There have been many changes described which are associated with acclimatization, but the exact relationship of these changes to the acclimatization itself has not been too well established

Fremont Smith That is what I meant.

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LYMPHATIC ADJUSTMENTS IN SHOCK*

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OUR STUDIES HAVE led us to believe that we can no longer disregard the rheology of the lymph when we discuss peripheral circulatory homeostasis Unfortunately this subject has had very little attention in experimental hemorrhagic or traumatic shock and generalizations have been based on observations of the most accessible pathways such as those in the cutaneous layers An increase in thoracic duct lymph flow in the dog has been reported during hypotensive shock caused by intestinal manipulation (1) and leg injury (2) However one article was found describing a marked and progressive diminution of subcutaneous lymph flow during hemorrhagic shock in the dog (3) Maintenance of effective or increased lymph flow in the thoracic duct during shock at a time when flow in the other lymph trunks diminished or ceased would seem to suggest either a different regulation of the terminal vascular bed of the alimentary canal or a different dynamic behavior of its lymph vessel system or both The terminal lymphatics have received little attention and have been assumed to form a static system of vessels

The constant occurrence of congestion of the abdominal viscera and especially of the alimentary canal during hemorrhagic or traumatic shock, at a time when the skin and striated muscles are rather ischemic, was the motivation for a study of the sequence of dynamic changes both in the blood vessels and in the adjacent lymphatic channels of the

*Aided by research grants from the National Institutes of Health, U S. Public Health Service (Grant H 79) the Josiah Macy Jr Foundation the Research and Development Division, Office of the Surgeon General, Department of the Army (Contract No DA-49-007-MD-388) the New York Heart Association and the Postley Fund. The skilled assistance of Jack Godach and Ira Forbes in carrying out these studies is gratefully acknowledged. Dr Baez is now in the Department of Anesthesiology New York University New York, N Y

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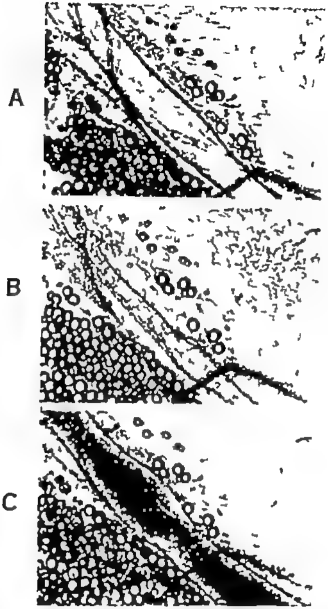

FIGURE 78 (*A*) Segment of a mesenteric lymphatic channel, photographed before the hemorrhagic experiment was begun, showing two open valves and regional terminal blood vessel crossing at two points in the vicinity of the valves. (*B*) *The same segment as in* (*A*) *after blood equal to from 2 per cent to* [illegible] per cent of the body weight had been withdrawn, showing intense narrowing of the lymphatic channel. Blood flow was still being maintained. Slight enlargement of venule is evident. (*C*) The same segment after spontaneous uptake of blood had begun but before forced infusion, showing atonic distention of the lymphatic channel with marked accumulation of erythrocytes. The flow in the terminal blood vessels was deficient. (Enlarged from 16 mm. motion picture film.)

rat mesentery The present report of these studies is concerned chiefly with the changes during hemorrhagic shock

Female rats of the Wistar strain weighing from 110 to 130 gm were used in these experiments Hemorrhagic shock was induced under light anesthesia by graded bleeding, controlled by a self infusion appa ratus as previously described (4) For direct microscopic observation of a representative lymphatic channel in the mesentery a short segment (2 inches) of jejunum or ileum was exteriorized through a midline incision. The gut was placed over a lucite block, held in place by cotton which was moistened by a constant drip of 1 percent Ringer gelatin solution at body temperature. The changes in sensitivity to the stimulus of topical epinephrine of both the regional lymphatic vessels and their accompanying terminal muscular blood vessels were deter mined throughout the hemorrhagic experiment. Changes in frequency and intensity of the contractions of lymphatic vessel walls and valves, as well as modifications in the manner of the lymph flow were noted

Cinematographic recordings of the blood and lymphatic vessels in the selected field were made at several representative stages of the shock syndrome by means of a special camera with a zirconium arc lamp A monocular microscope with a built in light meter was used at 32 and 60 times magnification

There are two types of lymph vessel wall contractions in the mesentery (*a*) peristaltic like waves of contraction which are initiated in the gut and progress rapidly toward the first lymph node (Such a contraction usually appears to the observer as an instantaneous total contraction of the lymph vessel wall. Its progressive peristaltic charac ter may best be demonstrated by high speed photography) and (*b*) par tial wall contractions which occur anywhere along the vessel wall [They are not progressive and are in general, intense and of brief duration (5)]

Before the hemorrhagic shock experiment, spontaneous contractions of the walls and valves of the mesenteric lymphatic channels (Figure 78 *A*) occur at a rate of from 8 to 12 per minute The lymph flow is continuous, with intermittent increases in flow coincident with partial wall contractions and particularly with peristaltic like waves The cell population is rather scanty and is essentially lymphocytic. Erythrocytes indicate a traumatized preparation which should be discarded The terminal blood vessels accompanying the lymphatics are in essence similar to those elsewhere in the mesentery Their only outstanding and constant feature is the presence of short A V or A A channels in the neighborhood of the valve sites sometimes describing a complete circle about the lymph vessel The normal threshold reactivity of the

lymphatic vessels to topical epinephrine is about 15 to 20 times lower than that of the terminal muscular blood vessels the metarterioles and precapillaries

Immediately after the initial bleeding of from 0 8 to 1.2 per cent of body weight the lymphatic vessel diameter (from 60 to 120 μ) remains unchanged. Both partial wall contractions and peristaltic waves of contraction become more frequent and intense The cell population increases considerably but remains leukocytic The sensitivity of the lymphatic vessels to epinephrine increases in the same proportion as that of the muscular capillary blood vessels *i.e.* from 1 to 5 times At this early stage after an initial slight narrowing of the terminal arterioles the blood vessels regain their normal pattern of blood flow with a rapid effective venous return. With the graded hemorrhagic procedure employed in our laboratory a distinct change in diameter of the lymphatic channels does not appear until from 2 to 2 2 per cent of body weight of blood has been withdrawn at about 1½ hours after the beginning of the experiment (Figure 78 *B*)

This change consists in a passive narrowing of the lumen to about one half its normal diameter Superimposed on this may be seen waves of active contraction which cause the lymphatic walls to approach each other further and then return to their semicontracted state Since the frequency of these active contractions is now much reduced the lymph flow becomes intermittent and formed elements (lymphocytes) are practically absent Vasomotion of the metarterioles and precapillaries now becomes maximally enhanced and their over all sensitivity to epinephrine increases from 10 000 to 50 000 times over control levels This hyperreactive adjustment of both the lymphatic and terminal blood vessels persists until a plateau of maximal bleeding is established averaging 3 5 per cent of body weight and occurring at about 2½ to 3 hours after the beginning of the hemorrhagic procedure The typical (6) series of decompensatory features then begins to develop in the blood vasculature At the same time atonic distentions of the lymphatic vessel wall occur in separate zones along its length with zones of constriction intervening so that a varicose appearance results. The lymph flow which almost ceased at the peak of the hyperreactive stage increases again slowly and cells reappear These however are now red cells which appear to be very sticky since they clump together in small numbers and form thrombi along the lymphatic vessel walls The beginning of this atonic lymphatic wall distention and the appearance of erythrocytes coincide with the initiation of spontaneous blood uptake from the reservoir As the amount of blood taken up increases the intestinal wall becomes progressively more edematous and con

gested Red cells continue to accumulate in the lymphatic vessels until eventually these vessels resemble greatly dilated veins (Figure 78 C) The spontaneous wall contractions are now scarcely perceptible and are incapable of forward propulsion.

At the end of the 2 hours of drastic (30 mm Hg) hypotension the remaining blood, which has not been spontaneously taken up from the reservoir is slowly infused and the blood pressure returns nearly to the control value. Although the large arteries and arterioles regain an almost normal diameter and flow the autonomous vasomotion of the metarterioles and precapillaries does not return and they are quite unresponsive to the threshold dose of topical epinephrine Patches of petechial hemorrhage are present in the endothelial capillaries Immediately following transfusion, an exaggerated motion is seen in the lymphatic vessels consisting of successive waves of peristaltic contractions which progress along the entire length of the vessels At this time the blood cells which accumulated in the lymphatic channel as it slowly distended before transfusion, are propelled forward and instead of the flow of clear lymph a frank blood flow occurs The site of this transvasation of blood from its natural compartment to the lymphatics must be the several layers of the alimentary canal. At present, however it was directly observed only at two levels of the gut wall, in the venular plexus of the submucosa and in the small terminal vessels of the muscular coat The duration of this transvasation of blood depends on the degree of gut congestion In cases of minor congestion of the gut it may not appear at all, and if it is present it tends to disappear again. In other cases the blood flow through the lymphatic vessels continues until exitus In the latter instance the flow in the blood vessels becomes sluggish and finally stagnates while the regional lymph vessels continue to show spasmodic contractions with an intermittent flow until exitus

Shorr Would you describe the association of the blood vessels with the valves?

Baez. I am glad you brought that up Dr Shorr for I believe that the small blood vessels which accompany the lymphatic channels of the alimentary canal deserve further attention Krogh mentions globoid bodies formed by a large number of small coalescent vessels in the submucosa of the intestine which Mall had described earlier and named "rete. He suggested that the strategic location of this vascular organization in close contact with the lymph vessels coming from the villi might facilitate further interchange of substances beyond that occurring at the villi (7) In the mesentery the site of our observations the architectural pattern of the accompanying vessels is less complicated

The constancy of this pattern affords a unique opportunity for studies on the comparative behavior of both systems of vessels and on the flow in both compartments Briefly it consists of one or two muscular metarterioles (from 16 to 28 μ diameter) which lie in close proximity to the lymph vessel wall Sometimes they are so close to each other that at low power distinction can be made between their walls only during the lymphatic vessel s active contraction Usually from 4 to 6 capillaries branch off and are then collected into one or two venules. These, after curving slightly run along again proximal to the lymphatic vessel One peculiar feature of this vasculature is the presence of A V or A A shunts which almost always occur at the valve sites Whether this has to do with the autonomous motion of the lymphatic valve or as suggested by Krogh (7) constitutes a last outpost of further exchange of solutes between the lymphatics and the blood vessels remains to be determined

Knisely If it were possible to measure the blood volume at this time what would it mean? Would the blood volume measured at this time be a number which could be counted upon exclusively in making intelligent interpretations of what is occurring?

Baez. Are you speaking of the dynamics of the lymph or of the reliability of the blood volume measurement?

Knisely Anything at all you want to do with it

Fremont Smith Are you trying to say we have to know what the volume in the lymphatics is to interpret the meaning of blood volume?

Knisely I should say that there are many alleged blood volume measurements which men are now trying to use to interpret experiments and understand phenomena, but that said alleged blood volume measurements probably do not mean anything at all and serve only to confuse our ideas

Fremont Smith Because of the blood cells in the lymphatics or the fluid in the lymphatics?

Knisely Both and fluid outside of the lymphatics and outside of the vessels too I agree with Dr Baez in this argument

Baez I believe any assessment of circulating blood volume in situations in which the normal permeability of the capillary bed has been disrupted to the point of frank extravasation of formed elements, such as we have just seen would require very cautious interpretation

I may add that I have extended these observations to include the effect of drum trauma There we found a different situation which is not as dramatic as this Immediately after a rat has been given 700 rotations in the drum, its blood pressure falls to 70 or 80 mm Hg When a portion of the gut which is usually congested is exposed for observa

tion, the majority of the lymphatic vessels appear atonic and distended and are quite unresponsive to epinephrine. Slow but continuous flow of lymph is present, with a mixed population of red and white cells The blood pressure declines steadily, and within 40 to 60 minutes is down to about 40 mm Hg If one attempts to raise the blood pressure by infusing about 1 to 1 5 ml. of blood, obtained from a donor rat there appears an immediate outpouring of blood into the lymphatic channels, giving them the appearance of large distended veins very similar to those seen in the hemorrhagic shock experiment

Fine This might be the place to mention Dr Frank s experiments on dogs to see whether by passing the liver by a total Eck fistula to prevent splanchnic congestion would affect the course of hemorrhagic shock (8) The Eck fistula did prevent congestion of the gut Nevertheless, the dogs died about as quickly as those without the fistula My suggestion is that perhaps the accumulation of red cells in these lymphatics is an uptake of extravasated red cells From the results on Eck fistula experiments, this phenomenon would not play a significant role in the course of the shock state. The red cells are returned to the circulation anyway

Baez I agree with you Dr Fine on the point that the red cells accumulated in the mesenteric lymphatics could only come from extravasated cells in the *wall* of the gut, and that this could only be a phenomenon of the splanchnic circulation. I am not in a position however to comment on blood that has been extravasated *within* the gut As far as we can tell from direct microscopic observation, most of the cells from the numerous foci of petechial hemorrhage which develop in the submucosa and the muscular coat during hemorrhage or after trauma find their way directly into the neighboring lymphatic capillaries At the present we have not been successful in making a satisfactory Eck fistula in the rat, which might help us to answer one of your points The closest that we have come to the studies you mentioned is our experience with the Eck fistula in the dog with the arterialized liver (9) These animals are not only resistant to a standard hemorrhagic procedure, with high mortality in the controls but they also maintain the hepatic ferritin systems intact and develop no gut congestion

It might be of interest to add that the prior injection of an adrenergic blocking agent (dibenzyline) in a dose which affords protection against hemorrhagic or drum shock also prevents congestion and transvasation of blood into the lymphatics

Srikantia What happens to the mesenteric lymph nodes?

Baez I can say only that they become very edematous and congested

The constancy of this pattern affords a unique opportunity for studies on the comparative behavior of both systems of vessels and on the flow in both compartments Briefly it consists of one or two muscular metarterioles (from 16 to 28 μ diameter) which lie in close proximity to the lymph vessel wall. Sometimes they are so close to each other that at low power distinction can be made between their walls only during the lymphatic vessel s active contraction Usually from 4 to 6 capillaries branch off and are then collected into one or two venules. These, after curving slightly run along again proximal to the lymphatic vessel One peculiar feature of this vasculature is the presence of A V or A A shunts which almost always occur at the valve sites Whether this has to do with the autonomous motion of the lymphatic valve, or as suggested by Krogh (7) constitutes a last outpost of further exchange of solutes between the lymphatics and the blood vessels, remains to be determined

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It might be of interest to add that the prior injection of an adrenergic blocking agent (dibenzyline) in a dose which affords protection against hemorrhagic or drum shock also prevents congestion and "transvasation" of blood into the lymphatics

Srikantia What happens to the mesenteric lymph nodes?

Baez I can say only that they become very edematous and congested

Fine In the dog when a plethora is deliberately produced by excessive transfusions, virtually all the blood that is put in escapes into the peritoneal cavity with a resulting tremendous hemoperitoneum and with no evidence of an overt vascular leak. It is a diapedesis caused by increased resistance to return flow in the liver This may have been operating in your rats on a smaller scale, and may have been showing up only by lymphatic uptake

Baez In our experience with dogs and rats frank *hemoperitoneum* occurs rarely after total replacement of the blood shed and when it happens we regard it as a result of a leak somewhere and we discard the *animal from our series* In our animals hemorrhage often occurs in the lumen of the gut and results in frank melena As a rule even a rat with extensively congested gut shows hardly a few tenths of a ml of clear or at most pinkish, fluid in the peritoneal cavity

Rappaport Dr Frank, how long after the formation of Eck fistula did you produce hemorrhagic shock?

Frank The animals had recovered from the operation and were in what we judged to be good health, and yet we did not want to let them remain as Eck fistula animals too long to deteriorate As I recall, it was 2 or 3 weeks

Furchgott Dr Baez, is the contractile motion of the lymphatics dependent on *innervation?* Also do the lymphatics contract on exposure to agents such as epinephrine and norepinephrine?

Baez I have not worked personally on the neurogenic control of *lymph motion but it has been reported (10) that electrical stimulation* of the peripheral end of the sectioned splanchnic nerve induced an enhanced contraction of the lymph vessels, while similar stimulation of the distal end of the cut vagus has been reported to give diverse results (10 11) These studies seem to require further work We have confirmed Florey s observation that wall contractions continue for a time in lymph channels which have been sectioned at both ends *This* led him to believe that the motion is independent of the central nervous system.

Both the muscular small blood vessels as well as the *lymphatic* channels under observation are responsive to the topical application or intravenous injection of epinephrine or arterenol The blood vessels are from 15 to 20 times more sensitive than the lymph vessels to the topical application of this agent while the reverse is true on intravenous injection

Fremont Smith What are you applying at that time?

Baez Epinephrine If it is applied topically to the mesentery in perceptibly

slow the blood flow in the capillaries by narrowing the metarterioles and precapillaries very slightly this threshold concentration will produce no response in the adjoining lymph channel If on the other hand, the concentration of epinephrine is increased so that within about 20 seconds of its application, the frequency and intensity of the contractions in the lymph channel increase then the circulation in the small blood vessels stops immediately as a result of the greater sensitivity of the latter vessels

Furchgott What I am uncertain about is whether you feel that the contractile activity of the lymphatics is myogenic or neurogenic in origin

Baez. The presence of nerve fibers and endings has been demonstrated by Carleton and Florey (12) histologically in lymph channels of about 150 to 250 μ in diameter However no mention was made of the valve sites I am inclined to believe that the nature of the contraction is myogenic, and basically governed by the physicochemical status of its immediate environment, but also possibly modulated by neurohumors

Frank We have had some cannulated thoracic ducts which show what you show so much more clearly that is the pinking of the thoracic duct lymph as shock progresses

Baez I believe that the reported differences in the dynamics of the cutaneous and the alimentary canal lymphatics is in accord with your observation May I ask whether you have noticed blood tinged lymph flow during your shock experiments?

Frank Yes there is very bloody lymph toward the end of the shock period

Shorr Dr Baez, in your control and aureomycin treated rats subjected to drum trauma, what is the time interval in which most of the animals will die?

Baez. In drum trauma the majority of the deaths ordinarily occur within the first 3 hours

Shorr I think this time interval has some bearing on the degree of invasion, or the multiplication of bacteria, and hence on their participation in the syndrome

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INDEX

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